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Gel-Diffusion Techniques for the Analysis of Parasitic Materials

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Various techniques for antigenic analysis of helminths have proven to be valuable in the immunology and serology of parasitic disease. The use of isolated parasite tissues and body fluids and the isolation of antigenically active chemical fractions and enzymes are examples of one avenue of research. Another technique has been the utilization of *in vitro* and adsorption procedures with living worms and metabolic product antigens. The gel-diffusion technique as a tool in antigenic analysis is relatively new. The method has been employed quite extensively by immunologists and bacteriologists for the past 10-15 years and has recently been applied in the analyses of several kinds of parasitic problems.

Agar diffusion methods have been used for many purposes: to study qualitatively and quantitatively the multiplicity of components in antigen-antibody systems; to determine the purity of materials, to quantitate and standardize different batches of reactants; to differentiate even such closely related proteins as ovalbumin and human albumin (Allison and Humphrey, 1960); to visualize the results obtained by chemical purification and fractionation procedures and to identify similar substances in different complex extracts. Since the rate of diffusion of an antigen in agar is a product of the molecular weight and concentration, fairly accurate measurements of molecular weight of proteins can be made by determining diffusion coefficients (Polson, 1958). The limiting factor in the use of agar diffusion is the fact that this is an immunological tool, and can only be used to analyze materials which are antigenic.

Gel-diffusion is essentially an extension of the precipitin test for analysis of antigen-antibody systems. In the familiar precipitin ring test one overlays antiserum with an antigen, producing a sharp interphase between the two fluids. In a positive test, a band of precipitate forms at the interphase. In the Ouchterlony test (simple agar diffusion) serum is solidified with agar or other gels (Moritz, 1960) and then overlaid with antigen. Antigen molecules diffuse into the serum and form precipitate bands with antibody molecules where the two reactants meet in optimal proportions. Early studies by Oudin (1952) with known mixtures of antigens against specific antisera have shown that each precipitate band which forms in the agar represents at least on antigen–antibody reaction. Since the diffusion rate of protein materials in gels is directly dependent upon the concentrations of the material and their molecular weights, proteins of different molecular weights in mixtures of antigens usually produce separate bands. It must be recognized, however, that the number of bands represent a minimum and not a maximum number of antigen-antibody systems since one band may mask other systems.

A variation of the tube technique is the double diffusion method of Oakley and Fulthorpe (1953). In this method a layer of agar is interposed between the antiserum and the antigen and the bands of precipitate develop in the clear agar as the two reactants diffuse toward each other.

Diffusion on a single plane as in a petri dish or on a slide was used by Ouchterlony (1948) for testing the toxin capacity of *Diphtheria* bacteria. By judicious arrangement of wells of antiserum and antigen and employment of the reagents at the proper concentration, bands of identity and non-identity forming in the agar between the reagents may be recognized. When two antigen mixtures containing a common antigen diffuse toward a well of antiserum placed at the point of an imaginary triangle between the three reactants, a curved continuous band of precipitate is formed by the common components; when the antigenic components are not identical the bands of precipiate are straight and cross each other. Ouchterlony (1958) has recently reviewed many of the developments of this type of double diffusion.

The gel diffusion technique lends itself to modification and alteration to fit particular situations. The combination of agar-diffusion and electrophoresis by Grabar and Williams (1955), is an example of an extension of the usefulness of the method by combining it with electrophoretic techniques. Techniques for drying and staining Ouchterlony reactions prepared on slides have been developed (Crowle, 1958). Micromethods have been used which require as little as 0.01 ml of serum or antigen (Preer, 1956; Crowle, 1960). We use a modification of three such techniques in our own work (Crowle, 1958; Grasset, Bonifas and Pongratz, 1958; Mansi, 1958).

Gel diffusion techniques have been used to study a number of problems in the analysis of parasitic antigens and immune phenomena. Wodehouse (1956), using Oudin's and Ouchterlony's techniques, analyzed changes in larval antigens of *T. spiralis* after heat, acid and proteolytic digestion. He also investigated the presence of common antigens in closely related and unrelated helminth species. With homologous antiserum prepared in rabbits against extracts of larvae, he found a minimum of 10 precipitin bands divided into an inner ring of 5 and an outer ring of 5. Heating the antigen to 58°C had no effect on the pattern of bands; heating to 100°C destroyed one prominent band in the outer group; autoclaving for 1 hour destroyed 9 bands. Digestion with trypsin destroyed all antigen of the inner group whereas digestion with pepsin destroyed all antigens except one of the outer group (the same antigen that survived autoclaving). The bands of the outer group were active in diagnostic tests with human serum. He also used the technique to standardize his trichina antigens.

Kagan and Bargai (1956) using the double diffusion technique of Oakley and Fulthorpe (1953) analyzed Melcher's acid-soluble protein larval antigen of *T. spiralis* and found 3 bands. Attempts to separate these antigenic components (Kagan and Norman, unpublished) by ethanol precipitation were not successful since agar diffusion assays showed that the material which was precipitated was immunologically identical with components in the supernatant. Peenen and Kent (1960) reported in an abstract that they extracted immunologically active protein complexes from *T. spiralis* and assayed their extracts by agar-gel double diffusion against sera from experimentally infected animals and natural human infections.

In 1957 both Soulsby and Kagan working independently of each other published on the antigenic complexity of *Ascaris* tissues as visualized by double diffusion methods. Soulsby (1957) reported that whole worm antigen

with homologous antiserum showed 9 bands; intestine, 14 bands, of which 8 could be removed by sheep erythrocytes (believed to be Forssman antigen), cuticle, 9 bands, and a polysaccharide whole worm antigen, 4 bands. Kagan (1957) reported that whole worm antigen had 8-11 antigenic components; muscle, 5-6 bands, enteric fluid, 10-14 bands, cuticle, 8 bands, and a polysaccharide antigen had 4 major and 4 minor components. Toxocara antigens cross-reacted with *Ascaris* whole worm antiserum. *T. canis* antigen produced 5 bands and *T. cati*, 9 bands. It is readily understandable why, with so many common antigenic components, the serological differentiation of infection with *Toxocara* in children is not readily made from infection with *Ascaris*.

Kagan, Jeska and Gentzkow (1958) used the agar technique for the assay of polysaccharide and tissue antigens prepared from *Ascaris lumbricoides* var. *suum*. In this study an attempt was made to deproteinize the whole worm antigen by several methods. After each procedure the material was lyophilized and reconstituted to its initial concentration and tested against the same antiserum by agar diffusion. The original antigen produced 5 bands when tested with whole worm antiserum. When the antigen was autoclaved, band #1 was destroyed. Subsequent treatment with trichloracetic acid and extensive extraction with chloroform and octyl alcohol or dialyses failed to change the antigenic picture. In each instance four antigen-antibody bands formed in the same spacial configuration as in the original material. These tests indicate graphically the stability of the carbohydrate-protein bands in *Ascaris* antigens. Polysaccharide extracts of isolated tissues of the worm were made and assayed in agar. From one to three antigenic components were isolated for muscle, cuticle or enteric fluid, but subsequent serological tests with *Ascaris* and *Toxocara* antisera made by the hemagglutination test indicated that no specificity was achieved (Kagan, Norman and Allain, 1959). Kent (1960) reported in an abstract the use of the Ouchterlony gel diffusion and agar-gel electrophoresis techniques to assay antigens isolated from *Ascaris lumbricoides*, which were active in serologic and skin tests.

Levine (1959) analyzed antigens prepared from the life cycle stages and adult metabolic products antigens of two schistosome species (*Schistosoma mansoni* and *Schistosomatium douthitti*) by the double diffusion method. He employed a variety of antisera prepared in rabbits against the life cycle stages of both parasites and sera from infected animals. Levine obtained a maximum of 7 bands with an infected snail antigen by double diffusion. Eggs and infected snail liver antigens showed 4 bands with homologous antisera. Many antigens produced bands with antisera against heterologous phases of the life cycle indicating common antigens in the various life cycle stages of the parasite. Tests with sera of infected animals showed with different antigens 1-2 bands. Smithers (1960) using the Ouchterlony technique tested sera of Rhesus monkeys infected with *S. mansoni* against antigens prepared from cercariae, eggs and adult worms. He reported 1 strong band with cercarial extract, 2 or 3 bands with adult worm extract, and 4 bands with egg antigen. Positive reactions appear in the sera of infected monkeys 4-6 weeks after infection.

Soulsby, Sommerville and Stewart (1959), Soulsby and Stewart (1960) and Soulsby (1960) reported their studies on the self-cure phenomenon in sheep infected with *Haemonchus contortus*. Serum taken from resistant sheep was examined by the Ouchterlony technique. When tested with heated larval antigen, 4 precipitin lines were found. The antigenic components of

these lines were identified by preparing antigens to metabolic products, exsheathing fluid and parasite tissue. The line near the antigen was produced by tissue antigen, the second line by tissue antigens and exsheathing fluid, the third line by exsheathing fluid and metabolic products, the fourth line by metabolic products. Analysis of serum obtained before and after administration of infective larvae established the fact that the self-cure phenomenon against *H. contortus* was initiated by an antigenic stimulus arising from the ecdysis moulting fluid of the third stage larval parasite. Tromba and Baisden (1960) using the micro-Ouchterlony method of Wadsworth (1957) and Crowle (1958) developed a diagnostic technique for stephanuriasis, the kidney worm infection in swine.

For several years we have been interested in the serology of hydatid disease. We examined the serologic activity of antigens prepared from hydatid fluid, scolices and membranes of *E. granulosus* and hydatid fluid, cysts and scolices of *E. multilocularis* in the flocculation and hemagglutination test and found differences in their activity, (Kagan, Norman and Allain, 1960a). We also published results that indicated that hydatid fluid antigen gave some nonspecific doubtful reactions with sera from patients with various collagen diseases and speculated that these results were caused by autoantibodies in the serum of these patients reacting with host proteins in the hydatid fluid. Later, using agar diffusion techniques, we were able to demonstrate strong reactions between *Echinococcus* antigens and antiserum prepared against normal liver of various host species (Kagan, et al., 1960b). This analysis has been extended in a study of the complexity of these antigens by double diffusion and Ouchterlony agar techniques. Antisera prepared against each parasite antigen and normal liver tissue from man, pig, cow and cotton rat were employed in this study (Kagan and Norman, unpublished). In the *E. granulosus* antigens at least 23 different antigen-antibody components were followed. Four of these bands were found to be of parasite origin, 6 of host origin and 13 bands were of undetermined origin. *E. multilocularis* antigens showed at least 27 bands which could be studied. Four of these bands were of parasite origin, 7 of host origin and 16 bands could not be identified. The complexity of these hydatid materials is indicated by the fact that only 6 parasite bands (2 from hydatid fluid and 4 from scolices) and 10 liver bands (6 from pig liver and 4 from cotton rat liver) could be identified and a total of 35 bands could not be identified since no common band with parasite or liver antigens could be obtained. We also have data that indicates that had we prepared antisera in more than two rabbits additional antigen-antibody systems would have been visualized. There is no reason to believe that other antigen-antibody systems were not identified because of inadequate concentration of reactants, masked lines, denaturation of our antigens by freezing and thawing and other technical reasons. The analysis of hydatid antigens in addition to emphasizing the complexity of helminth materials, also identified some of the antigenic components in hydatid fluid to be of host origin. This is not too surprising since the larval stage of this parasite is in a cyst surrounded by a permiable membrane within the body tissues of the host, and body fluids must enter the cyst.

Serum agar techniques have been used to unravel the antigenic complexity of free-living (Preer, 1959) and parasitic protozoa. Moore (1957) published preliminary studies with *T. cruzi* and a recent abstract by Krascheninnikow and Jeska (1960) has been published on the use of gel diffusion in studies of specificity of three species of *Balantidium*.

Gel-diffusion techniques have thus been employed in a variety of ways to clarify problems in antigenic complexity, immunity and serology. Antigenic complexity of ascaris, trichina and schistosome antigens has been investigated; assay of purification procedures for ascaris and trichina antigens have been made; standardization and identification of antigenic components in *H. contortus* and hydatid antigens has been reported; analysis of the immune reaction in sheep at time of self-cure and identification of the antigenic stimulus has been published and problems of species specific antigens and use of gel-diffusion in diagnosis have been reported. The method is not difficult, can be performed with small amounts of reactant materials, and does not require elaborate equipment. The technique is especially useful since analyses with complex mixtures of antigens can be made. Diffusion in gels has been used to advantage by parasitologists and should continue to gain wider acceptance.

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The Allocreadioid Problem with Reference to the Excretory System in Four Types of Cercariae*

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Embryology of the excretory system in cercariae has been studied in varying detail for species of many families of digenetic trematodes. Features of that system, especially the structure of the bladder wall and location of the primary pores, were utilized as fundamental characters in the taxonomic system proposed by La Rue (1957). However, those features are not adequately known for some families which he placed in the Allocreadioidea. Two such groups are the Lepocreadiidae and Acanthocolpidae. For that reason, the embryology of the excretory system was studied in a marine species representing each of those families and also in *Cercaria pomatiopsidis*, which,

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like the acanthocolpid species studied, is an ophthalmoxiphidiocercaria but develops in a freshwater rather than marine gastropod.

Recently, Cheng and James (1960) have described for *Crepidostomum cornutum* features that are in major conflict with the observations by Hussey (1941) on that species. Resolution of that conflict concerning a representative of the Allocreadiidae is necessary before the status of other families assigned by La Rue to the Allocreadioidea can be considered. For that reason, this investigation was extended to include two species of allocreadiid larvae, i.e., ophthalmoxiphidiocercariae developing in freshwater bivalves.

Measurements of cercariae are in millimeters and from spontaneously emerging larvae killed in hot sea water and measured under a freely floating cover glass.

Allocreadiid Cercariae

Embryology of the excretory system was observed in two species of allocreadiid cercariae developing in *Sphaerium* sp., one from southern Indiana and the other from the Tippecanoe River. The two species differed primarily in the shape of the stylets but their adults were not determined.

In both species, development of the excretory system (Figs. 1-4) is essentially as Hussey (1941) described for *Crepidostomum cornutum*. The primary excretory pores are in the body-tail furrow and disappear with the formation of an excretory atrium and a definitive excretory pore in the tail socket dorsal to the base of the tail. There is no evidence that the excretory system extends into the tail as described by Cheng and James (1960).

A Lepocreadiid Cercaria

The lepocreadiid cercaria may be the species that Martin (1938) described as the larva of *Lepocreadium setiferoides*. The definitive excretory pore is ventral and immediately anterior to the tail socket; the eyespots are located near the posterior end of the oral sucker; the main excretory ducts extend almost to the level of the eyespots before receiving anterior and posterior collecting tubules; and more flame cells were seen than reported by Martin, the maximum number observed by the writer being expressed by the formula $2\,[(3+5+4)+(5+5+5)]$. Measurements of nine specimens are: body 0.300-0.327 long, 0.114-0.131 wide; tail 0.670-0.774 long, 0.044-0.054 in maximum width; oral sucker 0.049-0.059 by 0.060-0.063; ventral sucker 0.048-0.054 by 0.046-0.049; forebody length 0.126-0.142; distance between eyespots 0.025-0.031.

Round to oval embryos with no indication of a tail bud have a pair of flame cells on each side with capillaries uniting anterior to the ventral sucker primordium to form a ciliated main collecting duct (Fig. 13). Each duct expands posterior to the primordium and opens postero-laterally on the dorsal surface. When the tail bud becomes evident (Fig. 14), the number of flame cells remains unchanged but the ducts come close together posterior to the sucker and then diverge to open laterally in the body-tail furrow without entering the tail proper. As the tail grows (Fig. 15), a longitudinal row of six conspicuous, transitory cells appears in the tail's axis. By that time, the primary ducts have fused to form the excretory bladder and its epithelium is evident, with additional cells anterior to the bladder apparently contributing to its epithelium as the bladder elongates with further development. When the primary pores become obliterated (Fig. 16), a sphincter appears at the posterior end of the bladder, followed by a small atrium at the body-tail junction.

In rediae of all sizes, the excretory pores are lateral and more or less equatorial (Fig. 12). From each pore, a convoluted duct extends a short distance and receives an anterior and a posterior secondary duct, each of which is joined by the capillaries of three flame cells; the excretory formula of the redia accordingly is 2 [(1+1+1)+(1+1+1)], as reported by Miller and Northup (1926) for the redia of *Cercaria setiferoides*.

An Acanthocolpid Cercaria

The acanthocolpid cercaria may be the one Martin (1939) described as the larva of *Stephanostomum tenue* but differs from his account of the species in several respects. The main excretory ducts are ciliated; spines around the ventral sucker are in two or three indistinct rows; and the stylet is 0.017 long and points antero-ventrally (Fig. 11). Measurements of six cercariae are: body 0.435-0.487 long, 0.074-0.086 wide; tail 0.656-0.834 long, 0.042-0.046 in maximum width; oral sucker 0.045-0.051 by 0.045-0.046; ventral sucker 0.045-0.049 in diameter; forebody length 0.171-0.190; distance between eyespots 0.012-0.017.

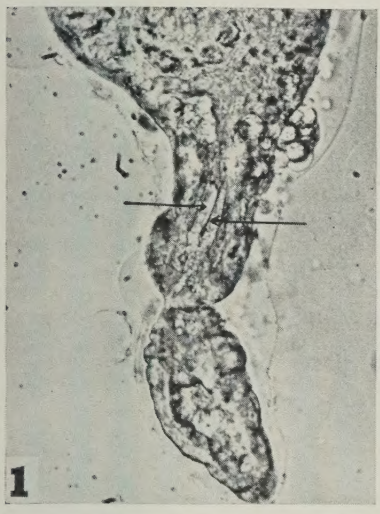
The young embryo has one pair of flame cells (Fig. 6). Before their ducts extend posteriorly to open, they curve anteriorly and thus anticipate the stenostomate pattern of the species. Additional flame cells appear before the tail bud is evident (Fig. 7). Shortly after it is differentiated (Fig. 8), there are six flame cells on each side and the main ducts begin to fuse behind the ventral sucker; they extend well into the developing tail and diverge to open laterally somewhat posterior to its midlevel. With the development of eyespots (Fig. 9), the primordial bladder epithelium appears as a mass of cells around the newly-formed bladder. With further development, the bladder extends to the ventral sucker and the caudal excretory tubules open laterally about one-fourth the length of the tail from its base (Fig. 10). In the fully developed cercaria, the bladder widens anteriorly, becoming slightly Y-shaped, and the large cells surrounding it occupy most of the space between the lumen and the body wall.

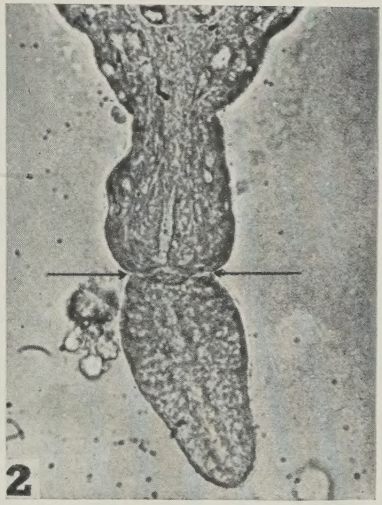
Very young rediae (Fig. 5) have an anterior and a posterior flame cell on each side with long capillaries uniting to form a short curved ciliated duct, which joins a convoluted vesicle opening at about the midlevel of the redia. The large redia has the same number of flame cells, but the capillary of each cell has a proximal recurrent loop.

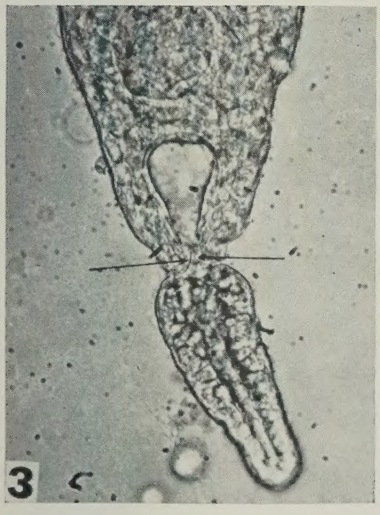
Cercaria pomatiopsidis (Stimpson, 1865)

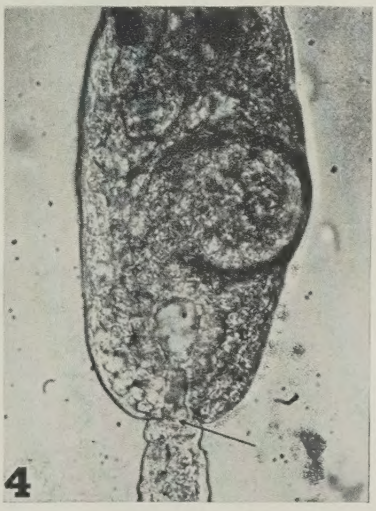
Cercaria pomatiopsidis is the only ophthalmoxiphidiocercous larva reported from freshwater gastropods of the United States. It develops in the amphibious snail *Pomatiopsis lapidaria*, which is widely scattered in the eastern half of the country, but the cercaria has been found only near Washington, D. C., by Stimpson (1865), in Michigan by Ameel (1939), and by the writer in Wisconsin. The stylet (Fig. 19) is 0.009 by 0.0035 and lacks the well developed alae characteristic of *Allocreadium* and *Crepidostomum* cercariae but its position in the oral sucker (Fig. 20) is similar to that of allocreadiid larvae.

Because material was scarce, the embryology of the excretory system was observed only to the extent that the primary pores were located in the body-tail furrow with the ducts seeming to extend very slightly into the tail (Fig. 18). No cilia were observed in the main collecting ducts which become somewhat convoluted just anterior to the ventral sucker before receiving the anterior and posterior secondary tubules.









Embryology of the excretory system in an ophthalmoxiphidiocercaria developing in *Sphaerium* sp.

Fig. 1. Embryo with primary ducts (arrows) not yet fused.

Fig. 2. Ducts fused to form primary bladder from posterior end of which ducts diverge to open at pores (arrows) in body-tail furrow.

Fig. 3. Primary ducts (arrows) posterior to bladder still evident and opening in body-tail furrow. Core of tail simulates a tubular extension of the excretory system with associated "epithelial" cells.

Fig. 4. Primary ducts posterior to vesicle fused to form excretory atrium (arrow) which lacks epithelium and does not enter tail. Bladder epithelium well defined.

The excretory pores of the redia are about three-fourths of its length from the anterior end (Fig. 17). From each pore a coiled duct extends anteriorly to near the midlevel of the redia to receive the anterior and posterior secondary tubules, each of which is much convoluted and joined by the capillaries of 7-9 flame cells.

DISCUSSION

The account of Cheng and James (1960) concerning the embryology of the excretory system in *Crepidostomum cornutum* contradicts not only the observations of Hussey (1941) on that species but also all other such studies that have been carefully made. Their description is lacking in details of that system in young embryos, particularly with respect to the primary pores and the relation of the main ducts to the primordium of the excretory epithelium in forming the bladder. That those features may have been observed is implied by their statement: "The entire process of excretory vesicle-formation conforms with that described by Sinitsin (1911) and pictured by Dobrovolny (1939) . . ." Cheng and James described the "vesicle" and its epithelium as growing into the tail to extend its length and open at the tip in a manner established for no other digenetic trematode. When living embryos are mounted and studied for some time, there is a gradual disintegration of tissues and displacement of cells which evidently misled Cheng and James. With slow death under those conditions, the core of the tail may become accentuated (Fig. 3) and resemble a tube with associated cells. There are many other instances in the literature in which the core of the tail probably has been mistaken for part of the excretory system.

Knowledge that a lepocreadiid cercaria has primary excretory pores in the body-tail furrow accords with the inclusion of the Family Lepocreadiidae in the Allocreadioidea of La Rue's (1957) scheme but the development of the excretory system in the acanthocolpid cercaria excludes its family from the Allocreadioidea as defined by him. The location of the excretory pores well posterior to the body-tail furrow and the stenostomate form of the system in the body are more in accord with the Echinostomatoidea of his scheme. Moreover, the stylet in the acanthocolpid cercaria is directed anteroventrally rather than dorsally as in allocreadioid larvae. Furthermore, Stunkard (personal communication) has recently found that *Cercaria dipterocerca* Miller and Northup, 1926, with an ornate tail but lacking a stylet, is unquestionably an acanthocolpid larva. That species and the present one are in agreement regarding the position of the primary excretory pores, structure of the bladder, and other features of the excretory system. Their adults seem certain to be species of *Stephanostomum* and the fact that one larva has a stylet and the other does not makes very questionable the phylogenetic significance of that structure in the Acanthocolpidae. Stunkard's observations thus remove a major difficulty in relating the acanthocolpids to the echinostome group, which they resemble in most features of adult morphology. Actually, the only basis for not including the Acanthocolpidae in the Echinostomatoidea is the embryology of the cercarial excretory bladder with which conspicuous cells are associated in the acanthocolpids but not in the echinostomes. Those cells give a thick-walled appearance to the bladder of several larval types, but as Cable (1956) has pointed out, their subsequent fate needs further study.

Cercaria pomatiopsidis differs from acanthocolpid cercariae in the position of the stylet and location of the primary excretory pores but agrees with the

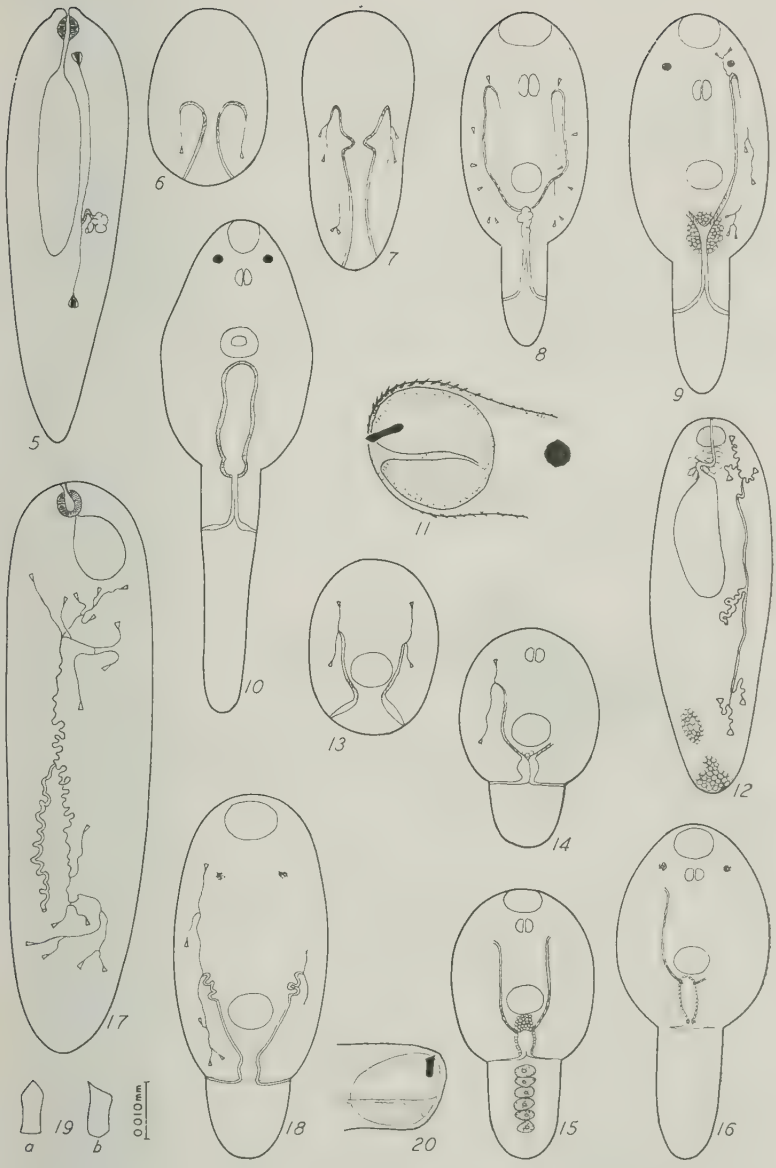


Fig. 5. Redia of an acanthocolpid showing excretory system of one side.

Figs. 6-10. Embryology of the excretory system in an acanthocolpid cercaria.

Fig. 11. Anterior end of an acanthocolpid cercaria showing position of stylet in oral sucker.

Fig. 12. Redia of a lepocreadiid cercaria showing excretory system of one side.

Figs. 13-16. Embryology of the excretory system in a lepocreadiid cercaria.

Fig. 17. Redia of *Cercaria pomatiopsidis* showing excretory system of one side.

Fig. 18. Embryo of *C. pomatiopsidis* with excretory ducts opening in body-tail furrow.

Fig. 19. Stylet of *C. pomatiopsidis* in (a) dorsal and (b) lateral views.

Fig. 20. Anterior end of *C. pomatiopsidis* showing position of stylet in oral sucker.

allocreadiid ophthalmoxiphidiocercariae in those respects. Ameel, Cort, and Van der Woude (1950) suggested that *C. pomatiopsidis* may be closer to the Allocreadiidae than to the Plagiorchiidae, on the basis of its germinal development. The present study supports that view.

SUMMARY

Embryology of the excretory system was studied in four types of cercariae included in the allocreadioid complex of digenetic trematodes. A conflict regarding that process in allocreadiid larvae (ophthalmoxiphidiocercariae developing in sphaeriid bivalves) is resolved by location of the primary pores in the body-tail furrow; at no stage of development does the excretory system enter the tail proper. A lepocreadiid (marine trichocercous) larva has primary excretory pores in the body-tail furrow and otherwise agrees with the Allocreadioidea. The marine ophthalmoxiphidiocercaria of the Acanthocolpidae has primary pores on the sides of the tail well posterior to its junction with the body. For that reason, the Acanthocolpidae is excluded from the Allocreadioidea, being perhaps closer to the Echinostomatoidea. *Cercaria pomatiopsidis*, an ophthalmoxiphidiocercaria developing in a freshwater gastropod, has primary excretory pores in the body-tail furrow and a vertically set stylet as in the Allocreadiidae.

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A New Species of *Pratylenchus* (Nemata-Tylenchida) from Roots of Soybeans

VIRGINIA R. FERRIS*

During a routine check of Illinois soybean breeding plots in the summer of 1958, large numbers of a single species of *Pratylenchus* were obtained from the roots of nine varieties of soybeans in one field. The males of this species often outnumbered the females and comprised from 10% to 50% of an entire collection (which usually contained many larvae as well as adults). Subsequent investigation showed it to be a new species. It is described herein as *Pratylenchus alleni* n. sp., after Professor M. W. Allen.

Specimens from the original collections were increased in the greenhouse on soybeans, wheat, and oats to provide material for study of its taxonomy and pathogenicity. Results of the pathogenicity studies will be reported elsewhere.

The description and drawings are based on studies of both living and preserved specimens. All measurements given are of specimens obtained from soybean roots. The nematodes were relaxed by gentle heat, killed and fixed in F.A.A., and dehydrated and mounted in glycerine using Thorne's (1936) methods.

Pratylenchus alleni, n. sp.

DIMENSIONS: 10 females: L = 0.38 mm. (0.33-0.44); a = 23 (19-27); b = 5.4 (4.7-6.1); c = 20 (15-25); v = $^{36}80^{4.8}$ ($^{28\text{-}48}78\text{-}83^{3.7\text{-}5.7}$); stylet = 14 microns (13.5-15).

10 males: L = 0.37 mm. (0.35-0.40); a = 26 (22-34); b = 5.3 (5.1-5.7); c = 20 (18-22); T = 45 (38-52); stylet = 13.6 microns (13.5-14.3).

Female (Holotype): L = 0.41; a = 22; b = 5.7; c = 21; v = $^{43}79^{4.3}$; stylet = 14 microns.

Male (Allotype): L = 0.35; a = 24; b = 5.2; c = 22; T = 52; stylet = 13.5 microns.

FEMALE: Lip region bluntly rounded, with 2 annules (one striation). Outer margins of sclerotized labial framework extend into the body about one body annule. The spear-guiding apparatus extends posteriorly from the basal plate of cephalic armature about 3 body annules. Both anterior and posterior cephalids present. The anterior cephalid is the larger of the two and is located at the second annule following the lip region. The posterior cephalid is located at about the 6th body annule. Stylet about 14 microns long. Basal knobs well developed, flattened anteriorly (Fig. 1, A). Hemizonid just anterior to excretory pore about 2 annules long. Ovary consists usually of a double row of oöcytes except for a short single row at either end. Spermatheca round, oviduct cellular from 1½ to 3 times as long as spermatheca, uterus usually about as long as spermatheca. Posterior uterine branch slightly longer than the width of the body at the vulva. Vulva-anus distance equal to about 3 (2.3-4.2) times tail length. Phasmids anterior to middle of tail. Two (and sometimes 3) of the 4 lateral lines extend past

*The author is indebted to Professor Gerald Thorne, Dept. Plant Pathology, Univ. of Wisconsin, Madison, Wisconsin, for his counsel and suggestions during the course of this work.

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phasmid. Tail without striations around terminus. Terminus usually with one or two indentations (Fig. 1, B) but sometimes smoothly rounded (Fig. 1, E).

MALE: Similar to female. Spermatocytes arranged in double or triple rows. Phasmids, slightly anterior to middle of tail, may or may not extend slightly into bursa. Spicula arcuate, hafted, resting on a simple, trough-shaped gubernaculum.

DIAGNOSIS: Small (0.4 mm.) *Pratylenchus* with two annules in lip region. Males numerous. Spheroid spermatheca present. Stylet about 14 microns long with anteriorly flattened knobs. Female terminus rounded. Most closely resembles *P. minyus* Sher & Allen, 1953, which Loof, 1960, considers a synonym of *P. neglectus* (Rensch 1924) Chitwood & Oteifa 1952. It can be distinguished from this nematode by its numerous males, by the spermatheca, and by the shorter stylet. It differs from *P. coffeae* by its small, stout body, shorter stylet, and round (never oval) spermatheca.

TYPE SPECIMENS: Holotype, female, from soybean root collection originally made in Eldorado, Illinois, on August 27, 1958, by R. L. Bernard, and increased in pot cultures in the greenhouse by the author. Slide *Pratylenchus*

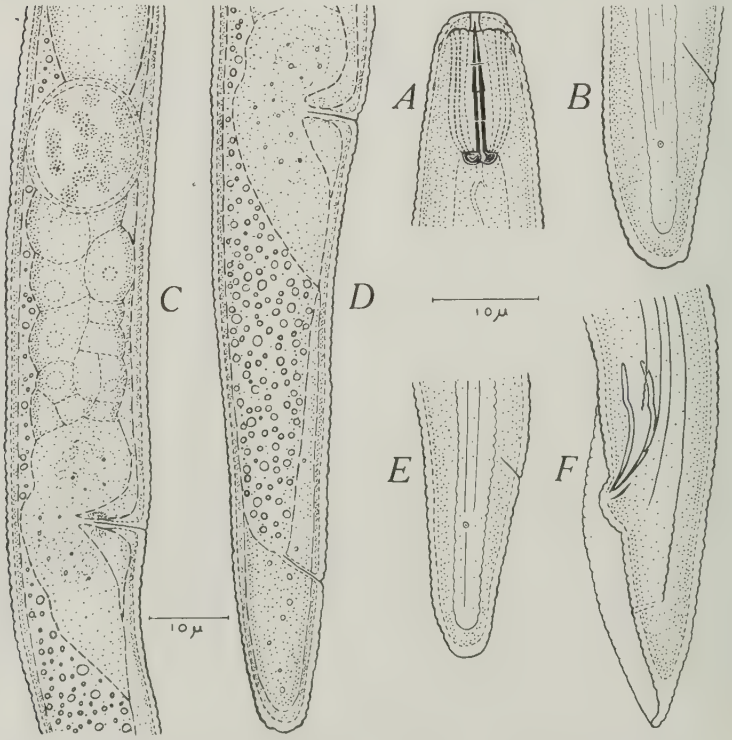

Fig. 1. *Pratylenchus alleni:* A, head of female; B and E, variations in female tails; C, part of female body showing vulva, uterus, oviduct, and spermatheca; D, posterior portion of female. F, male tail.

4. Dept. of Entomology Nematode Collection, Purdue University, Lafayette, Indiana. Allotype, male, same data as holotype, Slide *Pratylenchus* 4 a, Purdue Dept. of Entomology Nematode Collection. Paratypes, same data as holotype, 5 slides containing males, females, and larvae, deposited in Purdue Dept. of Entomology Nematode Collection.

TYPE HABITAT: Roots of soybeans (*Glycine max* (L.) Merr.).

TYPE LOCALITY: Soybean field 5 miles north of Eldorado, Illinois.

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A New Monostome, *Pleurogonius malaclemys*, n. sp. (Trematoda: Pronocephalidae) from Beaufort, North Carolina

WANDA SANBORN HUNTER*

Twenty-three adult Carolina diamondback terrapins, *Malaclemys terrapin centrata* (Latareille), 1802, were examined at the Duke University Marine Laboratory, Beaufort, N. C. Eight females (34.7%) carried a heavy infection of a pronocephalid trematode described herein. These trematodes were located in the posterior third of the small intestine; 7 turtles yielded from 50 to 135 worms each, but only 2 were present in one host.

The following description is based on study of many living and preserved worms which were killed in hot water without cover glass pressure. The measurements were made on 10 worms.

Pleurogonius malaclemys, n. sp. (Fig. 1)

Body elongate, relatively thick, with anterior end bluntly pointed and posterior extremity smoothly rounded. Cephalic collar inconspicuous; ventral lobes end somewhat less than one-quarter of body length, widely separated posteriorly, but joined medially about one-half distance behind oral sucker to form shallow concavity on anterior ventral surface. Concavity of entire body not pronounced in sections or in whole worms; sides of body mostly parallel between collar and extreme posterior regions.

Cuticle non-spinous. Entire body surface glandular and dark with scattered pigment, heaviest in anterior, collar and oral sucker regions suggesting remnants of eye spots, particularly in younger worms. Length varies from 0.707 to 1.78 mm. (av. 0.935). Width at posterior end of cephalic collar ranges from 0.265 to 0.441 mm. (av. 0.342). Oral sucker weak, subspherical, measures from 0.061 to 0.078 by 0.059 to 0.089 mm. (av. 0.065 by 0.075).

*Duke University, Durham, N. C., and the Duke University Marine Laboratory, Beaufort, N. C.

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Esophagus thin, long, without bulb, bifurcates sharply a little beyond one-fourth of body length. Caecae with many, closely-set diverticula on both medial and lateral surfaces throughout length. Diverticula smaller and more regular in anterior regions of cecae and with thick glandular walls, secretions in living worms often observed passing into gut lumen. Cecae diverge sharply to pass into lateral fields where they continue almost straight, depending on contractions, and posteriorly to anterior level of testes. Here they converge medially, continuing dorso-mediad to testes, to end near posterior extremity of body.

Lobate testes symmetrically located in anterior part of posterior body quarter, roughly triangular with bases posterior and lateral surfaces close to margin of body, averaging 0.171 mm. along anterior-posterior axis. Vas deferens formed immediately anterior to testes at preovarian level where it continues forward as external seminal vesicle. Seminal vesicle long with 3–5 conspicuous transverse loops dorsal to uterus and close to cirrus pouch. Cirrus pouch stout, undivided, and thin walled, measuring 0.965 to 0.058 mm, contains large basal prostate region and ejaculatory duct ending in small weakly armed cirrus. Cirrus pouch lies obliquely across long axis of body, primarily in dorso-ventral plane.

Ovary nonlobate, subspherical to oval in shape and lies to right of midline close to right testis; intertesticular, since its anterior margin seldom extends more than a few micra beyond right testis; average length is 0.0645 mm. Mehlis' gland lies on midline of body, para- and postovarial. Laurer's canal not observed.

Uterus mainly extracaecal and, anterior to testes, lies in closely-packed transverse coils, extending to equatorial line of body where it becomes intercaecal to genital pore. Metraterm weakly developed, one-half length of cirrus pouch, lying adjacent and posterior to latter.

Genital pores close together on body surface, to left of midline, ventral to left gut caecum a short distance behind bifurcation.

Vitellaria, two lateral masses of many small closely-packed follicles immediately anterior to and partialy overlapping testes. Vitelline ducts prominent and join to form a small reservoir ventral to oötype region.

Embryonated eggs numerous, measure from 0.029 to 0.034 mm. in length by 0.013 to 0.018 mm. in width (av. 0.032 by 0.016); polar filaments lacking.

Excretory pore muscular, subterminal and dorsal. Excretory bladder Y-shaped with very short stem; arms, however, wide and extend anteriorly to level approximately half way between ventral margin of cephalic collar and bifurcation of gut where they narrow sharply, turn mediad and unite. In living material, and in well-stained whole mounts, many irregular branches lead off from both medial and lateral surfaces of main collecting trunks and may or not anastomose.

Host: *Malaclemys terrapin centrata*
Site: Small intestine
Locality: Beaufort, N. C.
Holotype: U. S. N. M. Helm. Coll. No. 39052.

Discussion

The classification of the family Pronocephalidae has been one of great confusion which has resulted in a complicated synonomy, particularly in the study of the subfamily Pronocephalinae. Ruiz (1946) and Skrjabin

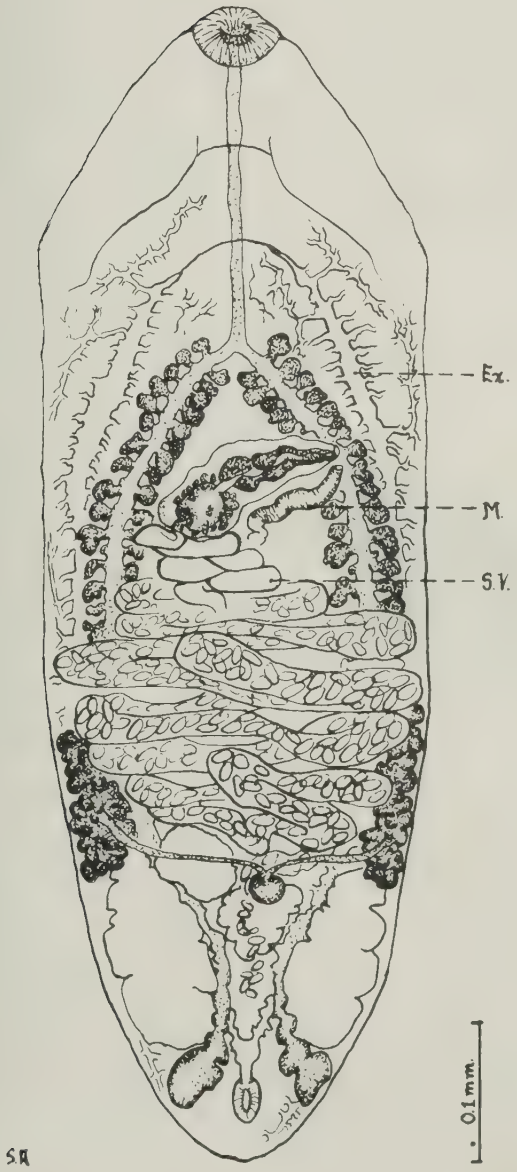

Fig. 1. *Pleurogonius malaclemys*, n. sp. Ventral view. Composite drawing made from photographs (Part of uterus omitted near metraterm). ex: excretory canals; m: metraterm; s.v.: seminal vesicle.

(1955) apparently are not in complete agreement on the synonomy. Skrjabin, the latest to attempt a revision, did not have some of the later descriptions of Caballero et al. (1954, 1955) or Pérez-Vigueras (1955). Yamaguti (1958) lists all described genera and species, notes synonomy, but presents no discussion. Using all available sources, however, the herein described form belongs in the generally accepted Genus *Pleurogonius*. In making a comparison with previously described species, it is interesting to note that many descriptions are based on a single, or at the most, very few specimens.

One generic character of the genus *Pleurogonius* is the pretesticular ovary. In this species, the bulk of the ovary is intertesticular even though it may extend slightly anterior to the testis when studied in sections. This one variation should not exclude this form from the genus. Also the numerous branches of the main excretory canals tend to set it apart. Gilbert (1938) states definitely that *Pleurogonius* does not have side branches on the main excretory tubes; his opinion is in agreement with Ruiz (1946) who separated the genus *Pleurogonius* from the genus *Pyelosomum* on this character. This author believes, however, that branches on the excretory canals may be found in other species upon examination of living specimens. A more important generic character is that the main lateral branches unite anteriorly in *Pleurogonius*, and in *Pyelosomum* they remain separate, turning posteriorly on each side at their anterior levels. Skrjabin (1955) apparently, according to figures shown, also considers this character of generic value.

The size of the worms has not been stressed, but they are strikingly smaller than all except for instances, *P. minutissimus* Looss, 1901, *P. bilobus* Looss, 1901, and *P. truncatus*, Prudhoe, 1944. The combination of specific differences such as position and relation of gonads, extent of uterus, size, shape and position of cirrus sac and metraterm, lack of polar filaments and size of the eggs, extremely glandular walls of the gut diverticula, and character of the excretory system, set *P. malaclemys* apart from all other described species of the genus *Pleurogonius*.

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Prodontorhabditis, n. gen. (Rhabditidae, Prodontorhabditinae n. subf.), a new soil nematode from East Pakistan

R. W. TIMM*

Many specimens of a small soil rhabditid were collected in June, 1960, from the rotting stem of a banana tree (*Musa paradisiaca* L.) on the grounds of Notre Dame College, Dacca. The nematode remained abundant throughout the rainy season. Males and females were equally numerous and both were active free-swimmers in water. The outstanding feature of this nematode was the presence of three small denticles at the base of the cheilostom. Although *Cheilorhabditis* and *Odontorhabditis*, discovered in exactly the same locality, have been described recently as having prominent mesostomal teeth (Timm, 1959), no genus of the Family Rhabditidae possesses teeth in a more anterior position. The presence of prostomal denticles and a valvulate esophageal bulb, together with the absence of a glottoid apparatus (metastom), necessitate the erection of a new subfamily of the Rhabditidae to accommodate the new genus. The name *Prodontorhabditis* is proposed for the new genus and Prodontorhabditinae for the new subfamily.

Prodontorhabditinae, n. subf.

DIAGNOSIS: Family Rhabditidae. Three-part rhabditid esophagus with valvulate terminal bulb; stoma with sclerotized cheilorhabdions and prostomal denticles; glottoid apparatus absent; male with leptoderan copulatory bursa supported by prominent genital papillae.

Type genus: *Prodontorhabditis*, n.g.

Prodontorhabditis, n.gen.

DIAGNOSIS: Stoma cylindrical, consisting of concave cheilostom, three prostomal denticles, and mesostome with cylindrical walls; glottoid apparatus absent; esophagus consisting of long procorpus, swollen metacorpus, narrow isthmus, and terminal bulb containing rhabditid valvular apparatus; ovaries amphidelphic, reflexed; testis single; equal cephalated spicules; gubernaculum parallel; copulatory bursa leptoderan; bursal papillae postanal; female tail filiform; male tail conical, ventrally concave.

TYPE SPECIES: *Prodontorhabditis pluvialis*, n.sp.

Prodontorhabditis pluvialis, n.gen., n.sp.

Measurements were made after gentle heat fixation in tap water. The measurements of the type specimens, however, are of glycerine mounts. Measurements: 10 females: Length = 0.58-0.74 mm. (0.63); a = 24.9-28.5; b = 5.3-6.2; c = 2.5-3.1; V = 37.5-43.5%; Ovl = 12.1-17.3%; Ov 2 = 14.1-16.3%. 10 males: Length = 0.34-0.45 mm. (0.42); a = 18-25; b = 3.5-4.2; c = 12.2-18. Holotype female: Length = 0.55 mm.; a = 25; b = 5.1; c = 2.8; V = 41.1%. Allotype male: Length = 0.36 mm.; a = 20; b = 4.3; c = 14.3.

DESCRIPTION: Body small and delicate, often bursting in fresh tap water. Fine transverse cuticular striation; striae about 1 micron apart; cuticle ending abruptly at head; distinct lateral lines about 2 microns apart, inconspicuous at anterior and posterior ends. Head slightly expanded but not set off

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The author is grateful to Dr. Bruce Hopper, Central Experimental Farm, Ottawa, for checking some of the literature not available to the author.

by constriction. Lips somewhat fused; inner circle of 6 tiny papillae; outer circle of 10 very fine setose papillae; amphids tiny, pore-like. Stoma long and narrow, 18 microns in male, 16-19 microns in female, consisting of: a distinct cheilostom, lined with lightly-sclerotized cheilorhabdions, wider than remainder of stoma and uniformly concave; a prostom, probably represented by 3 bluntly-rounded denticles, the subventrals being slightly more anterior and larger; a long heavily-sclerotized mesostom; glottoid apparatus lacking but metastom possibly represented by thickenings at base of mesostom; mesostom sometimes completely or partially collapsed. Esophagus consisting of a thickened procorpus extending to base of stoma, a swollen metacorpus, a narrow isthmus, and a valvulate terminal bulb. Esophago-intestinal valve cells spherical or flattened. Nerve ring crossing isthmus just above bulb, inclined ventrally. Excretory pore very inconspicuous, opposite anterior of bulb. Intestine in cross section consisting of 4 cells; lining of lumen wavy and often conspicuous; scattered olivaceous spheroids in intestinal cells. Female reproductive system amphidelphic; ovaries reflexed about 40% of distance to vulva; vulva lips slightly protruding; distinct spermatheca lacking; only one ovum in each uterus at a time, about 60 x 18 microns, up to 2-cell stage. Male with single slightly reflexed testis; spicules distinctly cephalated, deep brownish-yellow in color, 21 microns long or about 1.4 anal body diameters, with small anterior and lateral projections at tips; spicule tips not fused. Gubernaculum parallel to spicules, 12 microns long, consisting of two pieces joined distally to form a triangle. One pair of short subventral papillae immediately anterior to bursa; bursa voluminous, enwrapping tail, with a cleft at fourth pair of genital papillae due to inturned papillae; 7 pairs bursal papillae and 1 pair phasmids; pairs 3 and 5 turn outward and pairs 4 and 6 turn inward. Small lateral phasmids just behind pair 6 of bursal papillae. Female tail 16-20 anal body diameters long, gradually tapering to long filiform tip; anal lip protruding; phasmids a short distance posterior to anus. Male tail conical, ventrally concave, tapering uniformly to acute tip, 1.8-2 anal body diameters long. (One fourth stage male with genital papillae and lightly-sclerotized spicules had a long tapering tail, 11 anal body diameters long.)

HOLOTYPE FEMALE: Collected June 9, 1960; personal collection Slide No. S29.

ALLOTYPE MALE: Same data as holotype; No. S30.

PARATYPES (male and female): Nos. S31 to S33 and Vial 26.

TYPE HABITAT: Rotting stem of banana (*Musa paradisiaca* L.).

TYPE LOCALITY: Notre Dame College, Dacca, East Pakistan.

DISCUSSION: *Prodontorhabditis* seems to be closest to *Protorhabditis* (Osche, 1952) Dougherty, 1953, in which the cheilorhabdions are sometimes distinct and a glottoid apparatus is also lacking. The bursa, however, is peloderan in that genus and prostomal denticles are lacking. The stomatal denticles might be taken to indicate diplogasterid affinities, but even in *Pseudodiplogasteroides* Körner, 1954, which has a valvulate bulb, the esophageal structure is basically different and the valve is not of the "butterfly" type found in the Rhabditidae. Despite the fact that the Rhabditidae have recently undergone considerable fragmentation (Osche, 1952; Dougherty, 1955), no useful purpose would be served by trying to fit the new genus into one of the existing subfamilies.

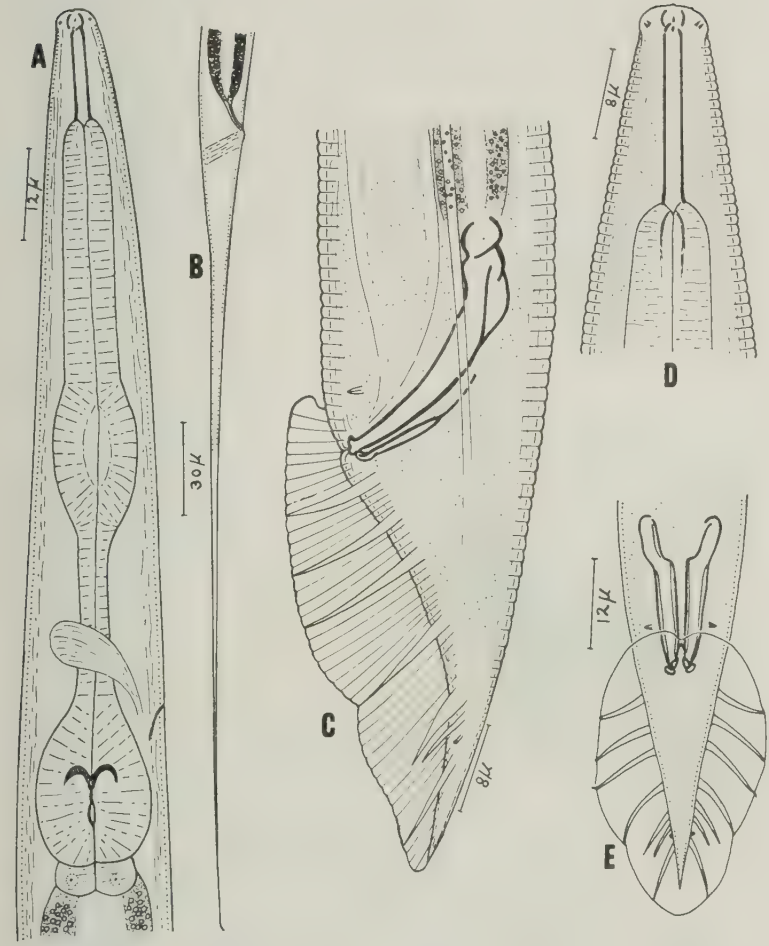

Figure 1. *Prodontorhabditis pluvialis*, n.g., n.sp. A. Esophageal region. B. Female tail. C. Male tail, lateral view. D. Female head. E. Male tail, ventral view.

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Morphology and Biology of the Genus *Plectus* (Nematoda: Plectidae)*

A. R. Maggenti

The genus *Plectus* Bastian, 1865 is one of the nematode groups of fundamental importance in the present classification of the Adenophorea (v. Linst., 1905), Chitwood, 1958. Because of the key position of this genus a study with emphasis on histologic morphology and biology was undertaken.

The species of *Plectus* are bacterial feeders and are among the so-called "free-living" nematodes. Nielsen (1948) reports that *Plectus* comprises approximately 68% of the nematodes found in moss and only 10% of those found in soil. Species of this genus have been collected from the Arctic to the Antarctic, and are reported from all major areas of the world, from sea level to over 14,000 feet above sea level.

I am particularly indebted to Dr. M. W. Allen for his encouragement, advice and interest throughout the progress of this paper. Appreciation is extended to Dr. B. G. Chitwood for his advice, and to Dr. W. Nicholas for the advice on the culturing of nematodes.

Materials and Methods

Histologic studies were made, for the most part, with females of *Plectus parietinus*. Specimens of *P. parietinus* used for serial sectioning were killed by gentle heat and then fixed in 2½% formalin for at least 24 hours. The processes of dehydration and dealcoholization were carried out in a depression slide. Xylene was employed to accomplish dealcoholization.

Chitwood and Chitwood's 1930 procedure for embedding was followed. Sections were microtomed at 5 microns. Pantin's (1948) procedure for regressive iron hematoxylin staining was used. Sections were stained with Baker and Jordan's (1953) modification of Heidenhain's (1896) iron hematoxylin. A 2-½% ferric alum solution was employed as a mordant and saturated solution of Picric acid in 95% ethyl alcohol as a destain.

Morphology

All specimens of the genus *Plectus* examined have six lips. There are interspecific differences in lip structure including size, shape, amalgamation and distinction from the general cephalic region.

Chitwood and Chitwood (1950) reported 12 papillae on the lips of *Plectus* in two circles, one external and one internal. I was able to see only six papillae, one medianly located on each lip (Fig. 1, A).

Four cephalic setae were present on all specimens of the species examined, two dorso-lateral and two ventro-lateral (Fig. 1, B). The flexibility of the cephalic setae is easily seen in living specimens and their innervations are visible in both totomounts and stained sections. Setae most commonly are located two or three annules behind the lips.

The amphids of *Plectus* have been described in the literature as unispiral or as shepherd crooks, apparently because the refraction of light through the amphidial pouch and tube creates this impression. The external form of the amphid is either a complete oval or a circle, depending on the species observed. The amphidial pouch is larger than its external opening (Fig. 1, C); it narrows rapidly at the posterior margin of the external opening into the amphidial tube. This tube is not symmetrically located but is shifted

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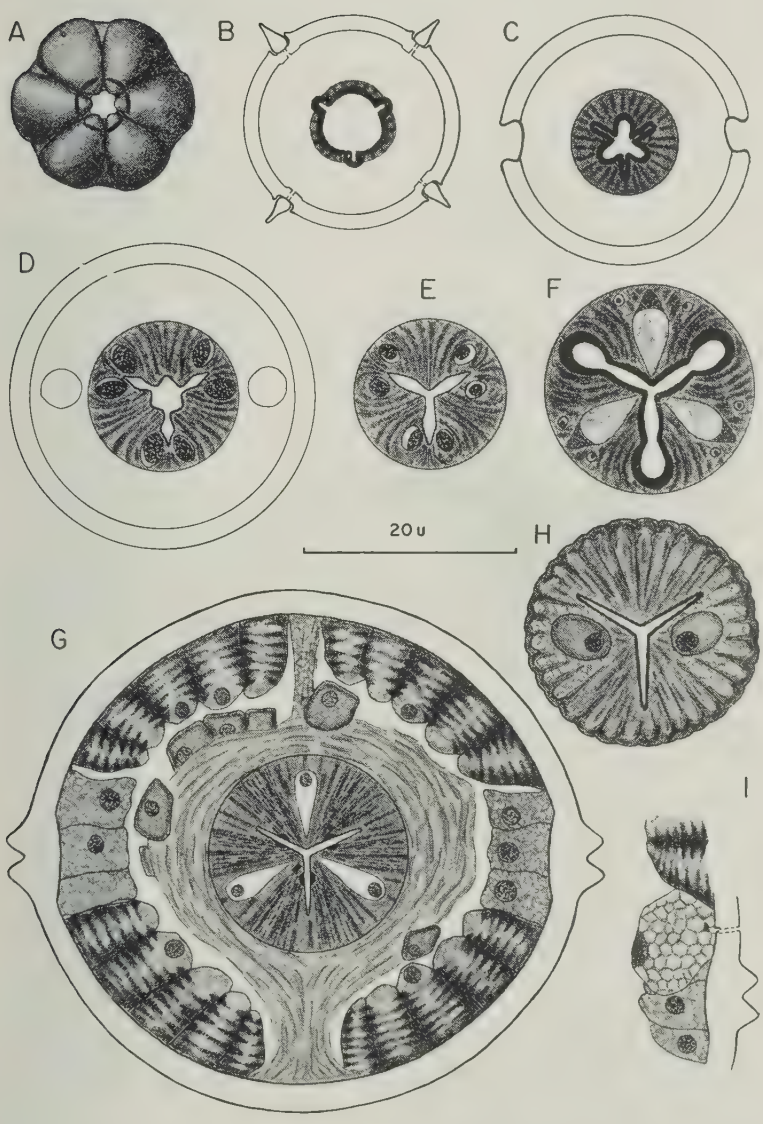

Fig. 1. *Plectus parietinus* female (all of equal magnification). A. Lip region, B. Prostom, C. Meso-metastom and amphidial pouches, D. Junction of stoma with esophagus, E. Fore-part of esophagus (corpus), simple triradiate lumen, F. Corpus of esophagus, lumen with radial tubuli, G. Cross section at level of nerve ring and "hemizonid," H. Anterior portion of posterior bulb, I. Hypodermal gland.

dorsally. The amphidial tube continues at the same diameter posteriorly to the sensilla pouch. The sensilla pouches are located in the region just posterior to the stoma (Fig. 1, D). Nerves leading from the sensilla pouches could not be followed. It is my opinion that the two subdorsal gland cells near the excretory or renette cell are the amphidial glands (Fig. 4, A and B, 1 and 2).

Cervical papillae (deirids) have been reported on *P. sambesii* by Andrássy (1958). They are present on all *Plectus* and located in or near the lateral longitudinal alae at the level of or just posterior to the nerve ring. They are also found to be present on the closely related genera *Anaplectus* and *Wilsonema*. *Plectus annulatus* has a single longitudinal ala with the cervical papilla dorsal to the ala. The cervical papillae are set into definite sockets and are innervated. The nerves could not be followed in either totomounts or stained sections. Chitwood and Chitwood (1950) state that in *Ascaris* each deirid is innervated by a branch of the nerve trunk which connects the medial externo-lateral ganglia with the nerve ring.

The external cuticle of *Plectus* species is marked by transverse striae which begin at the base of the lips and usually end approximately five annules anterior to the spinneret.

Lateral longitudinal alae occur as well defined ridges in the male and female. There are two alae on each side of the body in all known species except *P. annulatus* as noted above.

On longitudinal sections stained with Heidenhain's hematoxylin, transverse striae are evident in the trough between the non-striated longitudinal alae.

Males of *Plectus* do not have caudal alae. Preanal supplementary structures may or may not be present on *Plectus* males. Preanal supplementary structures when present consist of either preanal tubuli or a single preanal seta. The preanal tubuli are not always cuticularized or associated with glands. In some species a large sensory seta, short, but broad at its base, lies just anterior to the cloacal opening.

The body setae are not rigidly fixed but flexible. All have nerve connections and are set into sockets. Placement of setae on the body is variable. Usually there are three pairs of caudal setae on the female tail. The anterior pair are subdorsal, the next pair subventral and the most posterior pair subdorsal. The caudal setae of the male differ in appearance and number from those found on the female tail. On those males observed there were seven, eight or nine pairs of setae, the number depending upon the species. The caudal setae of males resemble the preanal setae. The males of some species also possess caudal papillae.

The hypodermis forms four chords, one dorsal, two lateral, and one ventral. The chords contain discrete cells, whose number varies from species to species.

Anteriorly the dorsal hypodermal chord of *P. parietinus* has a single row of nuclei. Posterior to the esophagus it is non-nucleated. The ventral chord contains a single row of cells and nuclei which persist throughout its length. The ventral chord contains the ventral nerve cord. Lateral hypodermal chords are composed of three rows of cells, two sublateral and one lateral (Fig. 1,G). The nuclei of the three rows are of equal size, and each cell is uni-nucleate. Near the tail region the lateral chords diminish in size but remain clearly defined to the middle of the tail. The thin non-nucleated portion of the hypodermis surrounds the body between the cuticle and the muscle sectors.

Hypodermal glands were not observed in all species of *Plectus*. In *P. parietinus* they are unicellular and occur sublaterally. Their number varies from specimen to specimen. The gland cells lie in the lateral chords, near the sublateral rows of cells, and open through sublateral pores in the cuticle (Fig. 1, I). Hypodermal glands begin in the esophageal region and continue posteriorly into the tail.

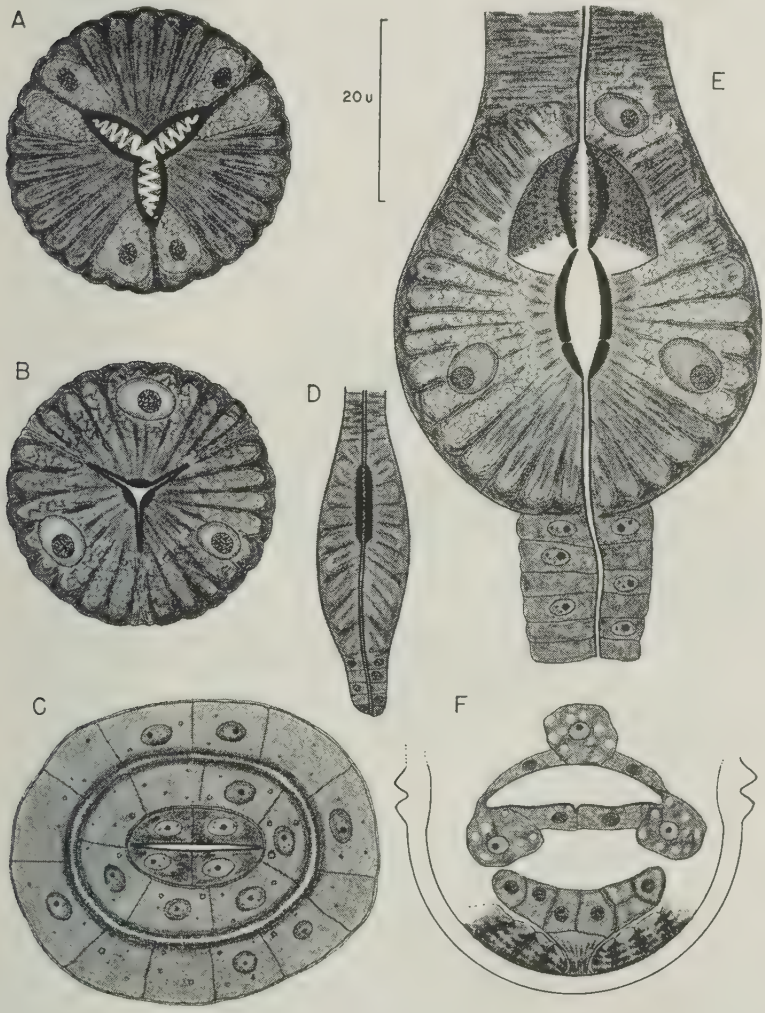

Fig. 2. *Plectus parietinus* female (all of equal magnification). A. Triradiate denticulated valve of posterior bulb, B. Posterior bulb at level of three posterior esophageal gland nuclei, C. Esophago-intestinal valve and intestine, D. Posterior bulb, first larva from egg, E. Posterior bulb, adult, F. Section through rectum and pre-anal ganglion.

In males and females of *Plectus* there are three caudal glands arranged in tandem which discharge their contents through a cuticularized spinneret. They are just posterior to the rectum or the cloaca.

The somatic musculature of *Plectus* is meromyarian. Each muscle cell is semi-coelomyarian (Fig. 3). Near the vulva there are 32 muscle cells. The exact number of cells in either the extreme exterior or posterior region of the body could not be determined. The nuclei of the muscle cells are large and are seen throughout the length of the animal.

The generic name *Plectus* was derived by Bastian from the odd loopings of the cuticularized excretory duct (Fig. 4, A) which opens ventrally just posterior to the region of the nerve ring. The lining of the duct is continuous with the external cuticle. Epithelial tissue surrounds the duct throughout its course, but nuclei were not associated with duct tissue. The excretory duct extends posteriorly to the excretory gland. It makes two loops after entering the gland, one on each side of the esophagus. The majority of the loopings are within the gland tissue (Fig. 4, A and B). The excretory gland is ventral, dorso-ventrally flattened, and extends laterally almost to the hypodermal chords. Its nucleus is large and centrally located. The long cuticularized terminal excretory duct is reminiscent of the Secernentea, as typified by the Rhabditids.

There are at least four coelomocytes in the body of *P. parietinus;* two are at the level of and subventral to the excretory gland (Fig. 4, A and B, 3). One and sometimes two occur halfway between the base of the esophagus and the anterior extremity of the reproductive system; if two, they are subventral, and if one, either ventral or subventral. The most posterior coelomocyte is located dorsal to the caudal glands.

The stomodeum of *Plectus* is divisible into three regions: stoma, esophagus, and esophago-intestinal valve.

The oral opening, which is immediately followed by the stoma, is formed by the joining of the lips and interlabial areas (Fig. 1, A). The stoma proper is divisible into three sections: cheilostom, prostom and meso-metastom. The cheilostom is hexagonal in cross section and heavily cuticularized. The six cheilorhabdions comprising the cheilostom are connected to the prorhabdions by a thin cuticular membrane (Fig. 1, A). The prostom is sub-circular in cross section. Short radial arms project into the body cavity from the two subdorsal areas, and the ventral region (Fig. 1, B).

The meso-metastom is distinctly narrower in diameter than the prostom, the radial arms are elongated, and the inter-radial areas are strongly convoluted (Fig. 1, C). Posteriorly the lumen of the meso-metastom decreases in size until it finally merges with the lumen of the esophagus (Fig. 1, D).

The esophagus is divided: corpus, isthmus, and posterior bulb. Posterior to the stoma the esophagus is muscular and has a simple triradiate lumen (Fig. 1, E). The first of the marginal nuclei appear in this region (Fig. 1, D.). There are six pairs of marginal (epithelial) nuclei in the corpus. Six pairs of radial (muscle) nuclei also occur in the corpus. The dorsal esophageal gland opens in the anterior region of the corpus. Posterior to the orifice of the dorsal esophageal gland, the radial arms of the lumen are modified distally by tubuli (Fig. 1, F). The major portion of the corpus is characterized by these radial tubuli.

The isthmus begins just anterior to the nerve ring. It is characterized by the complete absence of nuclei and a triradiate lumen without radial tubuli.

In the isthmus each radial arm is strengthened by longitudinal ridges persisting to the region of the posterior bulb. Muscle fiber attachments are concentrated at, and below, these ridges (Fig. 1, G).

The isthmus swells gradually to the heavily muscled posterior bulb. The triradiate lumen persists in the anterior portion of the posterior bulb but there are no longitudinal ridges on the radial arms (Fig. 1, H). The lumen expands just anterior to the triradiate denticulated valve. This valve is very conspicuous, and on its inner surface it bears several rows of denticle-like thickenings (Fig. 2, A and E).

There are 12 marginal, 12 radial, and five esophageal gland nuclei in the posterior bulb. According to Chitwood and Chitwood (1950) the order Chromadorida is characterized by the presence of three esophageal gland nuclei. However five esophageal gland nuclei have been seen in the genus *Plectus* in hematoxylin stained serial sections, a variety of totomounts, living specimens (with and without vital stains), formalin fixed specimens, and glycerine mounts. The esophageal gland nuclei are at two levels in the esophagus: two anterior to the denticulated valve (Fig. 1, H) and three

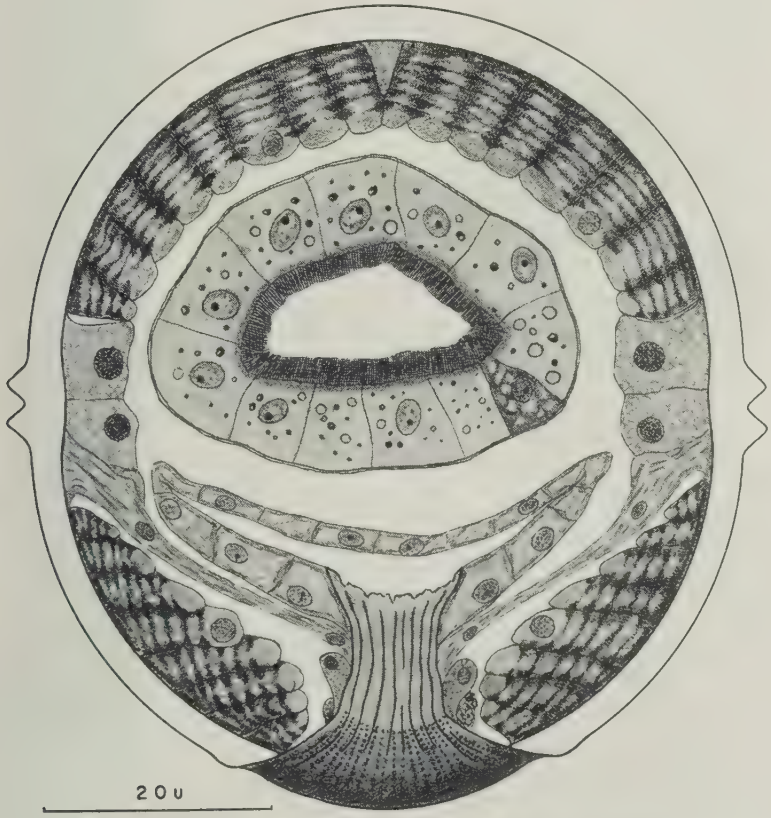

Fig. 3. Section through vulva and vagina, *Plectus parietinus*.

posterior to the valve (Fig. 2, B). The two anteriorly located nuclei are subventral. One of the three posterior nuclei is dorsal, and two are subventral. The orifices of the subventral glands are at the level of the nerve ring, and the orifice of the dorsal esophageal gland is near the base of the stoma. The lumen of the esophagus in the region of the three posterior glands is augmented between the radial arms by a thickening of the cuticular lining (Fig. 2, E).

Immediately behind the posterior esophageal gland nuclei the bulb tapers rapidly to the esophago-intestinal valve (Fig. 2, C and E). The cuticularly lined lumen in this region of the esophagus is triradiate without special modifications. The valve is dorso-ventrally flattened and composed of approximately 12 cells, and it invaginates the intestine.

The stomadeal nervous system consists of three longitudinal nerves, one dorsal and two subventral. The main nerves are contained in the esophageal sinuses, which run longitudinally in the three radial sectors of the esophagus (Fig. 1, F and G). According to Chitwood and Chitwood (1950) the stomodeal nervous system in *Ascaris* is connected to the central nervous system by the subventral stomodeal nerves; these enter into the esophagus at its beginning. This could not be confirmed for *Plectus;* it is assumed that the construction is the same or similar.

Morphologically the mesenteron of *Plectus* is polycytous and heterocytous. It is bounded by a thin membrane, the basal lamella. The cells of the mesenteron are uni-nucleate, columnar, and possess a bacillary layer (Fig. 3). A section parallel to the basal lamella shows the cells to be generally hexagonal.

The mesenteron is not divided into distinct regions. However, some histologic variations occur in the bacillary layer, stored food, and the shape of the lumen. Anteriorly and posteriorly the bacillary layer is low (Fig. 2, C): being higher in the remainder of the intestine (Fig. 3). The lumen is at first rather tubular; then, in the main region of the intestine highly irregular, and becoming triradiate just prior to the rectum. Stored food products and inclusion granules also diminish in the posterior region.

The mesenteron is heterocytous and two kinds of cells are present. The majority of cells probably function in absorption. The second kind are fewer in number and are found randomly throughout the mesenteron. These cells probably function as secretory cells. These cells and the nucleolus were more receptive to Heidenhain's hematoxylin than the cells presumed to be absorbers. Morphologically secretory cells are more vacuolated, and have fewer cell inclusions (Fig. 3). Absorbing cells and their nuclei stained lightly with hematoxylin (Fig. 3).

Between the mesenteron and the proctodeum there is an uninucleate sphincter muscle with a laterally placed nucleus.

The proctodeum in *Plectus* is the rectum. It is dorsoventrally flattened at the rectal-intestinal junction. The cuticle of the ventral wall is thicker than that of the dorsal surface which is curved and there is a visible fold in the cuticle along the lateral margins (Fig. 2, F). The ventral wall is somewhat flattened and has a median groove down its entire length (Fig. 2, F). It sweeps up at the lateral margins. The rectum is supplied with discrete epithelial cells which surround it to its posterior end.

There are three cells in addition to epithelial cells located at the anterior end of the rectum, one dorsal and two subventral. It is the author's opinion

that these cells are rectal glands. Rectal glands are a rarity among the Adenophorea but have been suspected before (Chitwood and Chitwood, 1950). These glands are at least three times the size of other rectal epithelial cells. Their nuclei are larger, and the endoplasm appears more vacuolated and stains a deeper blue with hematoxylin than that of associated cells (Fig. 2, F). The dorsal gland orifice appears to be in the dorsal wall of the rectum. The orifices for the subventral glands appear to be at the junction of the ventral and dorsal walls of the rectum.

The depressor ani muscle is of the usual "H" shape. This cell has posterior and anterior projections; the latter are attached on the rectum.

The female of *Plectus* has simple telogonic gonads with didelphic amphidelphic arrangement (Fig. 5, A). The reflexure occurs at the junction of

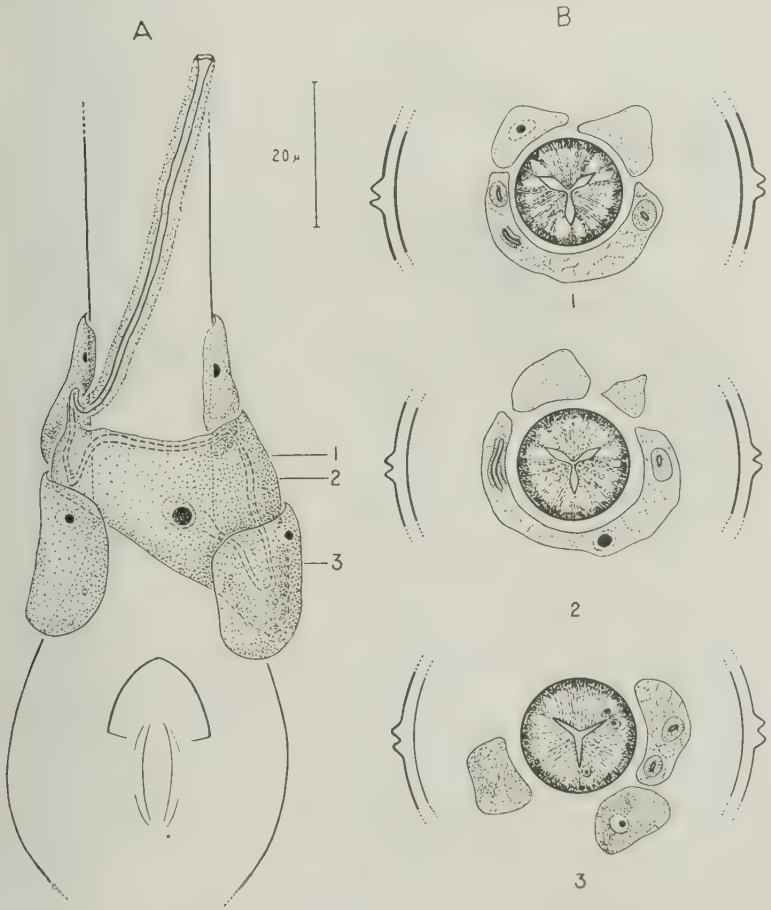

Fig. 4. *Plectus parietinus* (illustrations of equal magnification). A. Totomount illustration of excretory system and associated glands and coelomocytes, B. Cross-sections through excretory system: 1 and 2 subdorsal amphidial glands and excretory cell; 3 subventral coelomocytes and excretory cell.

the ovary and the oviduct; and the ovaries are reflexed in such a manner that the anterior ovary is on the right and the posterior ovary on the left side of the body. Both ovaries are pyriform, the germinal zone being at the narrow distal extremity and the growth zone at the broad proximal end. The oviduct is approximately one-fourth the length of the entire reproductive system and is followed by the uterus and vagina.

The ovary is covered by a thin synytial epithelium. There are no discrete cap cells at the distal end of the ovaries. The oogonia are at first regularly arranged with one cell terminal. As the oogonia develop the regularity is upset, and no particular plan can be discerned. Near the proximal end of the ovary a regular arrangement again prevails and the oocytes are placed alternately; this condition persists until an egg is developed. It then occupies the entire proximal end of the ovary. At the distal end of the oviduct there appears to be a network of muscle.

The syncytial epithelium terminates at the junction of the ovary with the oviduct. The oviduct consists of discrete, uni-nucleate, columnar epithelial cells. The cells are closely appressed and a lumen is not easily discerned unless an egg is passing through.

The uterus is distinguished by its increased diameter and by the tall columnar epithelial cells forming its wall. The proximal end of the uterus is distinguished by the lower height of the epithelial cells.

The vagina is supplied with squamous epithelium continuous with the epithelium of the body wall. The cuticle of the vagina is marked by striae and punctations. These markings do not cover the entire surface of the vagina but are concentrated near the vulva (Fig. 3).

There are four dilator vulval muscles, two anterior and two posterior. Each muscle contains four cells which are attached to the base of the vagina. From the vagina the muscles run obliquely, anteriorly and posteriorly respectively, to the lateral chords (Fig. 3).

The scarcity of males in the genus *Plectus* has made it impractical to study the reproductive system histologically. For this reason only the gross morphology can be discussed.

Males of *Plectus* have a reproductive system of the generalized type found in free-living nematodes (Fig. 5, B). Two testes (diorchic) are present and opposed.

The testes are covered with a syncytial epithelium like that covering the ovaries. The remainder of the reproductive system is covered with discrete epithelial cells which vary in size and arrangement according to the various regions they constitute (Fig. 5, B.).

In various species it was observed that the spicules are not symmetrical either in size or shape. The gubernaculum remained constant in appearance in any one species. The intestine runs between the head (manubrium) end of the spicules and joins into the cloaca near the distal end of the spicules.

The origin for the retractor muscles is in the region of the lateral chords. The insertion of the retractor spicule muscle is in the manubrial sinus on the latero-proximal surface. There are dorsal and ventral protractor muscles which are attached to the proximal end of the spicule. The ventral protractor muscle runs to the subventral body wall. The dorsal protractor muscle, however, appears to be inserted to the posteriorly directed dorsal process of the gubernaculum. The gubernaculum itself is supplied with muscles which run from the dorsal process to the ventral body wall. In addition to the

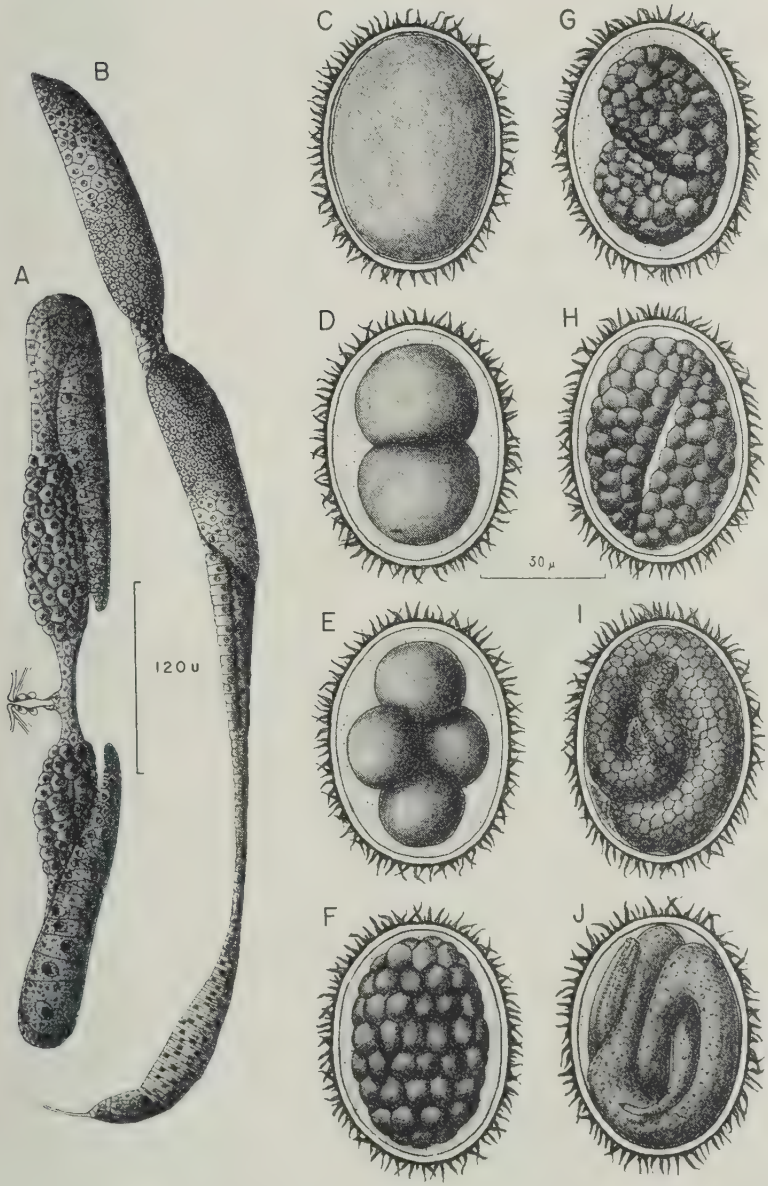

Fig. 5. *Plectus parietinus.* A. Female reproductive system, B. Male reproductive system, C. Single-celled egg, D. Two-celled egg stage, E. Four-celled egg stage, F. Gastrula, G. Embryo, beginning larval formation, H. Embryo in "tadpole" stage, I. Embryo pre-larval stage, J. Fully-formed larva just prior to hatching.

muscles of the spicules and gubenaculum, the male tail contains many accessory oblique muscles. These are attached to the lateral chord region and ventral body wall and are posteriorly directed.

Various components of the nervous system have already been discussed: setae innervations, cervical papillae and the stomodeal nervous system.

The main structure of the central nervous system is the circum-esophageal commissure, nerve ring, which is located around the isthmus just posterior to the corpus.

A structure is seen in totomount specimens which appears to be the same as the "Hemizonid" reported by J. B. Goodey, (1951) and described and identified by him in 1959. Stained sections show this "Hemizonid" to be that region where the circum-esophageal commissure extends to the ventral nerve cord in the ventral hypodermal chord, (Fig. 1, G). The totomount impression is created by the combination of the ventral extension from the nerve ring (ventro-lateral commissures) and the beginning of the ventral nerve cord.

The ventral nerve cord can be easily followed. Five major ganglia are recognized along the course of this cord. The first, the retrovesicular ganglion, occurs just posterior to the excretory cell, and anterior to the posterior bulb. A second is located just anterior to the vulva. The ventral cord proceeds posteriorly from this latter ganglia passing to the right of the vagina. Posterior to it there is a third ganglion. Two more ganglia are located in the posterior region of the body, one anterior (Fig. 2, F) and one posterior to the rectum. In addition to the major ganglia, minor ganglia occur along the ventral nerve cord and probably serve to innervate the somatic muscles.

BIOLOGY

MATERIALS AND METHODS: Cultures of *Plectus parietinus* were reared on Asparagine-mannitol agar (Thornton, 1922). The culture technique was modified and suggested by W. Nicholas (personal communication). A soil suspension was prepared by placing a teaspoon of soil in one liter of water, mixing well by shaking and then allowing it to stand a few minutes. From the suspension 0.2 ml. was pipetted off and placed on the agar plate (20 cc. of 2% agar in a four-inch petri dish). Plates were left at room temperature for three days in order to give the bacteria time to become established.

Experiments were conducted at 40°F, 50-55°F, 70°F and at a variable 70°F (room temperature), to determine the temperature most favorable to population increase. The temperature of 50-55°F proved to be the best of those tested, and subsequently cultures were kept at this temperature. Subculturing insured a continual supply of adults and eggs.

According to Nielson (1949) a major factor contributing to the death of eggs and larvae appears to be oxygen deficiency caused by rapid bacterial growth. This problem was minimized by constructing plastic frame well slides. The internal well measurements were 20 mm. × 20 mm. × 1 mm. deep. Agar was poured into the well slightly higher than the well depth. This allowed a free exchange of oxygen when a coverslip was placed over the well. Only the amount of bacteria transferred with eggs or adults from the stock culture was used to inoculate the slide. The slides were kept in petri dishes with moist filter paper. With this method eggs could be observed with the high dry objective of a compound microscope and in some instances even the oil immersion lens could be used.

Information concerning the life cycle of *Plectus parietinus* is derived from the experiments carried out at 50-55°F using these plastic frame well slides.

DISCUSSION: As with most free-living genera of nematodes, there is no special complexity of the female reproductive system. This is correlated with the slow production of eggs and also with the relatively few eggs produced at one time. Seldom are more than two fully developed eggs found in the uterus of *Plectus,* and when two are present, normally one egg is the product of the anterior ovary and the other the product of the posterior ovary. However, there are exceptional cases where several eggs are retained in the uterus.

The usual time between the appearance of the eggs in the uterus and egg-laying is three days. Normally eggs are laid in the single cell stage (Fig. 5, C). The cleavages of the egg are holoblastic and spiral. The first cleavage of the single cell is transverse; two distinct cells are formed (Fig. 5, D). The second cleavage is longitudinal, both the anterior and posterior cell dividing (Fig. 5, E). Twenty-four hours after the single cell egg is laid it has developed to an eight-celled form; 48 hours following this latter stage the egg has developed to a mature gastrula (Fig. 5, F). During the next 24 hours the embryo begins larval formation (Fig. 5, G).

Forty-eight hours after the first signs of larval formation, the embryo has lengthened considerably and is in the "tadpole" stage (Fig. 5, H). From this stage onward the embryo can be observed to move about within the egg shell. Lengthening of the "tadpole" continues until, at the end of ten days, the embryo is distinctly in the pre-larva form (Fig. 5, I). Fourteen days after egg formation the larva is fully developed (Fig. 5, J). Hatching occurs two to four days later.

It was not determined with certainty whether or not the larva molts once before hatching. When the larva first leaves the egg it differs morphologically from the adult in the form of the posterior bulb of the esophagus. In the first larva the posterior bulb is only slightly swollen, and the valvular apparatus consists of three denticulated longitudinal plates (Fig. 2, D). The expanded valve characteristic of all other larval stages and the adults of *Plectus parietinus* is absent (Fig. 2, E). For this reason it is believed that the first molt does not take place within the egg. The first apparent molt takes place approximately seven days after hatching and the posterior bulb and valve of this stage is like the adult.

Development from egg to adult female requires approximately 45-55 days at 50-55°F, and 60 days until eggs are again laid. Nielsen (1949), working with two species of *Plectus*, reported for one 20-25 days at 20-22°C and approximately 20 days for the other species at 15-16°C.

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The Development and Morphological Variation of *Philophthalmus gralli* Mathis and Leger, 1910 with a Comparison of Species of *Philophthalmus* Looss, 1899*

HILDA LEI CHING**

Looss (1899) created the genus *Philophthalmus* for *P. palpebrarum,* a non-spinous trematode with a very large pharynx, found in the eyes of birds in Egypt. Braun (1902) emended the generic diagnosis to include *Distomum lucipetus* Rudolphi, 1819 which has a spiny cuticle and a pharynx smaller than the oral sucker. Braun also described *P. lacrymosus* from *Larus maculipennis* from Brazil. Looss (1907) described *P. nocturnus* from birds in Egypt. Mathis and Leger (1910) described but did not include a figure for *P. gralli* from poultry in Tonkin. Sugimoto (1928) redescribed *P. gralli* from chickens and named *P. anatinus* from ducks in Formosa. To date, 21 species have been described from domestic and wild birds. One species, *P. lacrymosus,* has also been reported from man (Markovic, 1939) and Dissanaike and Bilimoria (1958) described *Philophthalmus* sp. from a man in Ceylon. Fisher and West (1958) found *Philophthalmus* sp. in the belted kingfisher and green heron in Indiana. At least four specific descriptions are based on single specimens: *P. indicus, P. nyrocae, P. occulare,* and *P. problematicus. P. skrjabini* was described from immature specimens.

Members of the genus are found in the eyes of vertebrates except for *P. coturnicola* and *P. skrjabini* located in the small intestine and *P. offlexorius* in the oral cavity of birds. Skrjabin (1947) named sub-genera of *Philophthalmus* on the basis of types of vitellaria but these are not recognized by Yamaguti (1958).

Fisher and West (1958), West and Fisher (1959), and Alicata and Noda (1959, 1960) described the life cycles of two unspecified members of the genus *Philophthalmus.* Both are similar to the life histories of species in a closely related genus, *Parorchis avitus* Linton, 1914 and *P. acanthus* var. *australis* Angel, 1954 determined by Stunkard and Cable (1932) and Angel (1954), respectively. The sexually-mature worms of these two genera have, in their uteri, eggs containing oculate miracidia in which mother rediae are already developed. The cercariae which are derived from daughter rediae are of the megalurous type with spiny cuticles, well-developed digestive tract, and tail with invaginated tip. They encyst readily on objects to form metacercariae which are infective upon ingestion by the vertebrate hosts. However, Alicata

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and Ching (1960) gave experimental evidence that cercariae and metacercariae of a species of *Philophthalmus* in Hawaii can infect the eyes of mammals by the ocular route and of birds by both the oral and ocular routes.

Alicata and Noda (1960) described the life cycle of the *Philophthalmus* species which was used in this study. Mother and daughter rediae were studied in experimental infections of *Tarebia granifera*. Cercariae encysted quickly on finger bowls forming pear-shaped cysts. The metacercariae were fed to chicks and ducklings and adults were recovered from the eyes after 4 weeks. The writers concluded that the trematodes resembled *P. gralli* Mathis and Leger, 1910. In this paper their trematodes will be referred to as such and the placement in this species justified.

Materials and Methods

The trematodes were studied from excysted metacercarial stage to the sexually-mature adult from experimentally-infected ducks, chickens, rats, and rabbits. Adults were obtained by several methods of infection as described by Alicata and Ching (1960): by placing cercariae and excysted metacerariae directly in the eye, and oral placement of encysted and excysted metacercariae. Cercariae were obtained from naturally-infected *Tarebia granifera* and allowed to encyst on finger bowls; excystment was accomplished by heating dishes upon which cysts adhered.

All measurements given are in millimeters.

Observations

The growth of *P. gralli* is described from specimens recovered from experimentally-infected chickens after excysted metacercariae had been placed directly in their eyes. The ages given are from the time of infection to the time the worms were removed from the hosts. In single dose infections worms did not develop at the same rate, particularly if the infections were heavy. Measurements and descriptions of organs in adult *P. gralli* have been reported by Mathis and Leger (1910) and Alicata and Noda (1960).

Ten recently-excysted metacercariae killed with hot water measured 0.458-0.550 by 0.113-0.160. Ten stained and mounted adult worms recovered 35 days after infection measured 3.263-3.969 by 0.102-1.411.

The cuticle of recently-excysted metacercariae is completely covered with small spines. Spination is sharply reduced in 10-day-old specimens, especially at the posterior ends of the body. Specimens at 30 days have scattered spines anteriorly on the ventral surface of the body, where they are most concentrated near the genital pore.

After 10 days, oblique, circular and longitudinal muscle fibers were observed at the anterior end of the body. Above the acetabulum, the body is usually active, whereas the broadly rounded posterior end is relatively inactive.

The flame cell pattern in the cercaria is 2 [(3+3+3) + (3+3+3)] (Fig. 1). The arrangement of collecting ducts and flame cells is similar to that of the larval form of *Parorchis avitus*. However, the adults of *Philophthalmus gralli* have a simple excretory system without the ramifications and anastomoses of *Parorchis avitis*. In *Philophthalmus gralli*, the ascending collecting ducts are enlarged with slight infolding at the anterior end of the ducts (Fig. 2). The tubules are ciliated and follow a course similar to the cercarial stage.

The oral sucker and acetabulum are of equal size in the cercaria, whereas in the sexually-mature adult the acetabulum has well-developed muscle fibers and is the same size or up to one-third larger than the oral sucker. The

esophagus and prepharynx are shortened in the adults and the pharynx, small and oval in the cercaria, becomes round and almost the size of the oral sucker. The acetabulum, near the middle or slightly posterior in the cercaria is in the anterior third or fourth of the adult.

The germinal cells in the cercarial stage form a longitudinal median mass anterior and posterior to the acetabulum. In 10-day-old specimens, the genital pore is observed equidistant to the oral sucker and acetabulum, just ventral to the intestinal bifurcation. Ducts can be seen leading from the genital pore and are probably the cirrus and uterus. The testes and ovary lie in a vertical line posterior to the acetabulum, with the testes closely opposed and twice the size of the ovary. In specimens older than 10 days, the genital pore continues to be located ventral to the intestinal bifurcation. The ovary is a small round mass located medianly. The testes, located in the posterior third or fourth of the body, are oblique or tandem and in the same size ratio to the ovary as in young specimens. They are nearly round with slightly flattened opposed sides. The edges of the gonads may be smooth but in specimens older than 35 days, the edges are often slightly lobed. In stained specimens two months old, the testes are sometimes reduced in size, stain poorly, or are missing. Of 58 specimens from 16-67 days old, 47 had smooth testes, 8 slightly lobed, two strongly lobed and one lacked a testis.

In mature flukes, the seminal vesicle may be elongate or globular. In older specimens, sperms are sparse in the seminal vesicle. The seminal vesicle varies in its location depending on whether the cirrus extrudes from the cirrus sac. It was posterior to the acetabulum in 54 specimens when the cirrus was enclosed. In three specimens with the cirrus extruded, the seminal vesicle within the sac was lateral and slightly posterior to the acetabulum. In one specimen with cirrus extruded, the seminal vesicle extended posteriorly to the midacetabulum. In specimens with most of the seminal vesicle posterior to the acetabulum, this organ was vertical to the sucker except in two worms where it appeared to be located diagonally.

Spermatozoa are found in the initial part of the uterus as early as 10 to 15 days and sometimes fill a large coiled portion of the uterus. Laurer's canal was found in preserved and live specimens with the duct directed dorsally towards the anterior testis. In mature specimens, the coils of the uterus increase, fill the body space from the anterior testis to the acetabulum, and overlap the ceca. The metraterm, muscular and sinuous, is located alongside of the cirrus sac before joining it at the genital pore.

The uterus is filled with eggs in different stages of development: those just formed, larger ones containing developing miracidia, and those containing fully-developed miracidia with eye spots. As the young miracidia develop, the eye spots darken, increase in size, and become fused. The eggs containing miracidia are larger at one end and are not operculated.

The vitellaria are tubular with several variations. The glands may appear as long tubes containing homogenous yolk material, tubes containing follicular material, or sinuous tubes (Fig. 3). Although the yolk material may be concentrated into follicles within the tubes, the vitellaria do not consist of clearly separated rounded masses. The vitellaria are single tubes found along the outer edge of the ceca and unite just posterior to the ovary. Their extent is 79-89% from this site anteriorly to the posterior edge of the acetabulum with the length of the glands on each side almost equal.

After a study of over 50 specimens of mature worms from four different hosts, the following characters were considered as fairly constant: location

of the genital pore, ratio of transverse diameters of suckers, ratio of diameters of ovary to testes, type of vitellaria, extent of vitellaria, extent of seminal vesicle, and egg sizes. Characters which varied included the arrangement and shape of testes, location of the acetabulum, and the lengths of the prepharynx and esophagus.

Variations observed due to age were: the size of the seminal vesicle, extent of uterine loops, condition of the testes, and spination of the body. In stained specimens older than 35 days, the testes were often the same size as the ovary and poorly stained; they may become irregular in shape and strongly lobed, and one testis may even be degenerate or missing.

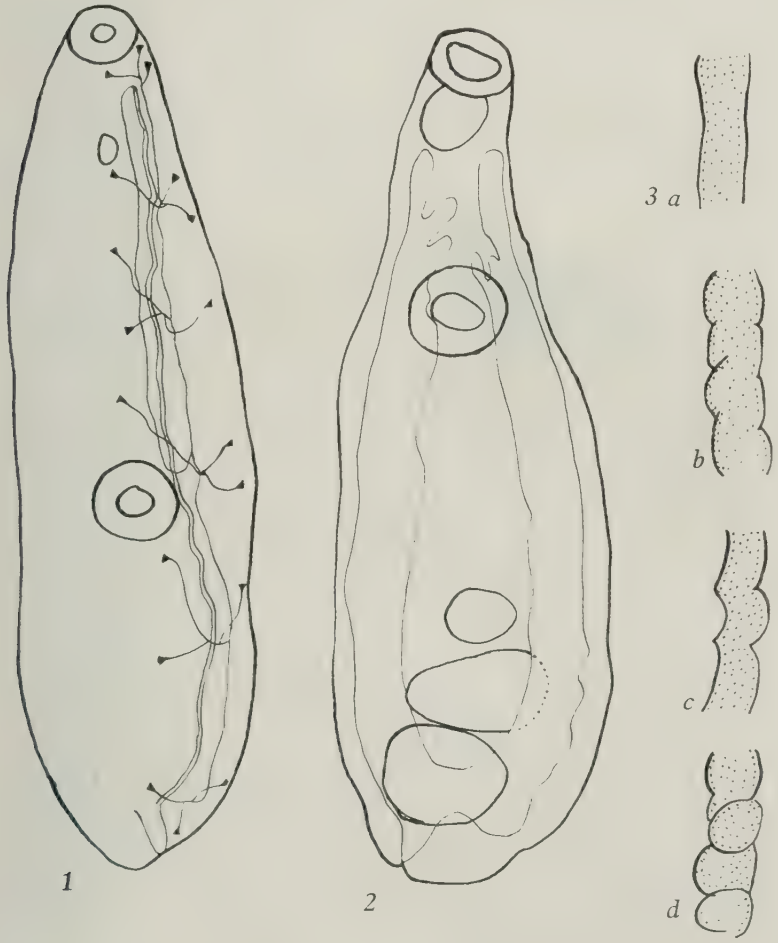

Figure 1. Freehand sketch of flame cell system in cercaria of *P. gralli*.
Figure 2. Freehand sketch of excretory system in adult.
Figure 3. Variations in vitellaria.

Variations due to the host were studied. Trematodes in the chicken appeared to reach the largest size in the shortest time. At 25-28 days, the size range and average of 10 specimens from the chicken was 2.47-4 by 0.661-1 (3.122 by 0.851) as compard to 10 specimens from the rabbit: 1.76-4 by 0.441.1 (2.571 by 0.719).

In the duck, chicken, and rabbit, the worms were commonly attached to the conjunctiva by means of the acetabulum. In the rat, worms were seen on the edges of the eyelid or on the surface of the eye.

The condition of the gonads seemed to be most affected by the type of host. Sixty-eight worms 22-35 days old from three hosts had the following characteristics:

Character	Host			
	Chicken	Rabbit	Rat	Total Worms
Gonad size ratio 1:1	0	3	1	4
Testes strongly lobed	0	1	1	2
Testes degenerate	1	0	4	5
Gonad missing	2	1	2	5
Gonads normal	40	10	2	52
Total worms	43	15	10	68

Worms from the rat most often had testes that were strongly loved, degenerate, or missing than worms of the same age in other hosts. Four specimens had one testis missing but one specimen lacked an ovary.

COMPARISON OF SPECIES

Characters regarded as fairly constant shall be discussed with reference to species descriptions available. Seventeen descriptions were available of 21 species named. Some information about the other four species, *P. aquillai, P. cupensis, P. hovorkai,* and *P. posaviensis* was obtained from *Helminthological Abstracts.* Comparisons were made from measurements and descriptions when possible; otherwise figures in the text were used. Hosts, localities and authors of known species are included in Table I.

LOCATION OF THE GENITAL PORE: The location of the genital pore has been noted in relation to the oral sucker, acetabulum, and intestinal bifurcation. Since the suckers and digestive system would vary in location depending on the degree of contraction of the worm, the location of the genital pore would vary correspondingly. Ten species have been described with the genital pore at or near the intestinal bifurcation and between the suckers. The genital pore of *P. sinensis* is between the pharynx and acetabulum and posterior to the intestinal bifurcation. The genital pore of *P. coturnicola* and *P. muraschkinzewi* is also posterior to the intestinal bifurcation but is located between the acetabulum and intestinal bifurcation. In *P. occulare, P. mirzai, P. indicus,* and *P. aquillai,* the genital pore is located anterior to the intestinal bufurcation.

RATIO OF TRANSVERSE DIAMETERS OF ORAL SUCKER TO ACETABULUM: Suckers of six species are almost equal: *P. coturnicola, P. mirzai, P. nyrocae, P. occulare, P. problematicus,* and *P. sinensis.* The sucker ratio of the other 11 varies from 1:1.3 to 1:1.8 in *P. anatinus, P. gralli, P. indicus, P. lacrymosus, P. lucipetus, P. muraschkinzewi, P. nocturnus, P. offlexorius, P. palpebrarum, P. rizalensis,* and *P. skrjabini.*

RATIO OF SIZES OF GONADS: The majority of species has testes two to three times the size of the ovary. *P. occulare, P. indicus,* and *P. coturnicola,* have ovaries slightly larger than the testes. *P. lacrymosus* and *P. problematicus* have gonads approximately the same size. Ratios of transverse diameters of the ovary to testes are:

1:0.08	1:1.5	1:2
P. coturnicola	*P. muraschkinzewi*	*P. anatinus*
P. occulare		*P. gralli*
P. indicus	1:3	*P. nyrocae*
	P. lucipetus	*P. offlexorius*
1:1	*P. mirzai*	*P. palpebrarum*
P. lacrymosus	*P. nocturnus*	*P. sinensis*
P. problematicus		*P. skrjabini*
		P. rizalensis

TYPE OF VITELLARIA: The yolk glands of this genus are either tubular or follicular. Species with distinctly rounded vitellaria, five to six follicles on each side, are: *P. lucipetus, P. lacrymosus, P. offlexorius,* and *P. skrjabini.*

Table 1. Known Species of *Philophthalmus* Looss, 1899

Species	Host	Locality
Philophthalmus anatinus Sugimoto, 1928	Duck	Formosa
P. aquillai Jaiswal, 1955	*Aquilla rapax*	India
P. coturnicola Gvosdev, 1953	*Coturnix coturnix*	Russia
P. cupensis Richter, Vrazic & Aleraj, 1953	Goose	Yugoslavia
P. gralli Mathis & Leger, 1910	Duck	Formosa
	Chicken	Indo-China
	Fulica americana alai	Hawaii
P. hovorkai Busa, 1956	Goose	Yugoslavia
P. indicus Jaiswal & Singh, 1954	*Neophron percnopterus*	India
P. lacrymosus Braun, 1902	*Larus maculipennis*	Brazil
	Casmerodius albus egretta	
	Larus ridibundus, Man	Beograd
P. lucipetus (Rud., 1819) Braun, 1902	*Larus fuscus, L. glaucus*	Vienna Museum
P. mirzai Jaiswal & Singh, 1954	*Milvus govinda*	India
P. muraschkinzewi Tretiakowa, 1946	Duck	Yugoslavia
P. nocturnus Looss, 1907	*Athene noctua*	Egypt
	Circus aeruginosus	W. Siberia
P. nyracae Yamaguti, 1934	*Nyroca ferina ferina*	Japan
P. occulare Wu, 1938	*Passer montanus taiwanensis*	China
P. offlexorius Mamaev, 1959	*Tringa incana*	E. Siberia
P. palpebrarum Looss, 1899	*Corvus cornix*	Egypt
	Milvus parasiticus	Europe
P. posaviensis Richter, Vrazic & Aleraj, 1953	Goose	Poland
P. problematicus Tubangui, 1932	Chicken	Philippines
P. rizalensis Tubangui, 1932	Chicken	Philippines
P. sinensis Hsü & Chow, 1938	Duck	China
P. skrjabini Efimov, 1937	*Larus ridibundus*	Russia
Philophthalmus sp. Dissanaike & Bilimoria, 1958	Man	Ceylon
Philophthalmus sp. Fisher & West, 1958	Belted Kingfisher	
	Green Heron	Indiana, USA

Other species have tubular vitellaria which vary to some degree. In *P. nyrocae,* the vitellaria are tubular with short branches. In *P. gralli,* the vitellaria are tubular with a small number of follicles, according to the original description. The vitellaria of *P. occulare* are "tubular outside of intestine and are in the form of scattered follicles inside," according to the description but the figure given indicates the opposite conditon.

EXTENT OF VITELLARIA: Measurements of the extent of vitellaria from the anterior testis to the acetabulum were made of 17 species from figures published. Extent of vitellaria:

90-95%	80-85%	70-75%
P. indicus	*P. anatinus*	*P. mirzai*
P. coturnicola	*P. gralli*	*P. muraschkinzewi*
P. palpebrarum	(of Sugimoto,	*P. nocturnus*
P. problematicus	1928 & Alicata	*P. occulare*
P. nyrocae	&Noda, 1959)	
P. sinensis		

55-60%	35-45%
P. lacrymosus	*P. lucipetus*
P. skrjabini	*P. offlexorius*
	P. rizalensis

EXTENT OF THE SEMINAL VESICLE: The extent of the seminal vesicle was noted in relation to the acetabulum. Those species with a seminal vesicle that reaches only slightly posterior to the acetabulum are: *P. coturnicola, P. indicus, P. problematicus,* and *P. sinensis.* Species with a seminal vesicle completely posterior to the acetabulum are *P. palpebrarum* and *P. rizalensis.* Other species have a greater part of the seminal vesicle posterior to the acetabulum.

According to Mathis and Leger (1910) the seminal vesicle of *P. gralli* is posterior to the acetabulum and transversally disposed. Sugimoto (1928) figures *P. gralli* with a vertically placed seminal vesicle.

EGG SIZES: Because the eggs in one worm may range in size from those newly formed to those containing miracidia, the measurements given in the species descriptions may be confusing unless the development of the eggs is also indicated. Egg sizes would also differ in fresh and preserved specimens. Two species, *P. palpebrarum* and *P. nyrocae,* are described with miracidia lacking eye spots. Hsü and Chow (1938) observed in their specimens of *P. sinensis* that eye spots may be lacking in the miracidia if they are still immature. *P. palpebrarum,* the type species, has the smallest eggs, 0.054 by 0.031, of those from the 15 described species. *P. nyrocae* described by Yamaguti (1943) from one specimen had collapsed eggs, 0.090 long.

Largest egg sizes are quoted for *P. gralli* by Mathis and Leger (1910) from fresh specimens shed by the worms: 0.158 by 0.070.

DISCUSSION

While the majority of the specimens of *P. gralli* was uniform in the important characteristics listed, variations occurred which warrant caution in naming new species. The reproductive organs were found to vary most due to age and type of host. For this reason, the shape of the testes, size of the seminal vesicle, and extent of uterine loops should not be used to distinguish

on species from another. A number of specimens is needed to determine the proportions of the gonads.

Spination of the cirrus and the body has been used as a difference among species. Four species are described with spines on the cuticle, 10 are unarmed, and seven descriptions do not include this characteristic or are unavailable. Most probably, the young adults of all species have spines which may decrease and disappear with maturity. Spines may be difficult to determine as in *P. gralli* where they were observed in some specimens on the ventral side of the anterior end only.

Skrjabin (1947) placed three species with follicular vitellaria in the subgenus *Philophthalmus* and five others with tubular vitellaria in the subgenus *Tubolecithalmus*. Variations in the type of vitellaria in specimens of *P. gralli* in Hawaii and in other speces have been discussed. At the point where the vitellarian ducts join, the yolk material is continuous and tubular in most species. As the vitellaria extend anteriorly towards the acetabulum, they may vary so that it would be difficult to determine whether they are basically follicular or tubular. The establishment of subgenera on the basis of this character is needless.

P. gralli was described by Mathis and Leger (1910) from birds in Tonkin. They recovered 26 parasites from 422 birds examined. No figure of the species was given but the trematodes were said to resemble *P. nocturnus*. According to the authors, the cirrus pouch occupies a transverse position posterior to the acetabulum. Alicata and Noda suggested that their specimens from Hawaii closely resembled those of *P. gralli*. Except for this location of the seminal vesicle, they are similar.

P. anatinus Sugimoto, 1928 and *P. nyrocae* Yamaguti, 1934 also resemble *P. gralli*. Sugimoto (1928) recovered *P. anatinus* from ducks and *P. gralli* in chickens in Formosa. His figure of *P. anatinus* appears in reality to be only an extended specimen of *P. gralli*. Differences between the species such as the general shape and curvature of the body, sucker ratio, and type and origin of the vitellaria are slight. The sucker ratios of the figures are the same although his measurements indicate that of *P. anatinus* to be 1:1.3 and that of *P. gralli* to be 1:1.7-2. Egg sizes of both species reported in Formosa are considerably less than what Mathis and Leger (1910) indicated.

According to Yamaguti (1934), the single specimen of *P. nyrocae* resembles *P. anatinus* in general anatomy but differs in that the miracidia lack eye spots. The vitellaria are located more anteriorly, 93% than in my specimens of *P. gralli*, 78-86%.

P. occulare Wu, 1938 resembles *P. indicus* Jaiswal and Singh, 1954 in the location of the genital pore, sucker ratios, gonad ratios, and egg sizes. However, *P. indicus* is twice as large as *P. occulare*. The vitellaria of *P. indicus* are elongated follicles extending 91% to the acetabulum while *P. occulare* has both tubular and acinous vitellaria that extend only 75% to the acetabulum.

P. coturnicola Gvosdev., 1953 and *P. problematicus* Tubangui, 1932 have similar sucker ratios, type and extent of vitellaria, gonad ratios, egg sizes, and location of the seminal vesicle. However, the location of the genital pore and the location in the host differ. *P. coturnicola* is one of two species found in the intestine.

P. nocturnus Looss, 1907 and *P. mirzai* Jaiswal and Singh, 1954 have identical locations of the seminal vesicle, suckers, and vitellaria. The size ratio of gonads is similar but the shape of the testes is different, being lobed

in *P. nocturnus*. The genital pore in *P. mirzai* is located anterior to the intestinal bifurcation.

The many similarities of the species in the genus indicate a need for further evaluation of each species as to the variations produced by age and host and differences in life cycles. Species which the present author considers valid are: *P. gralli, P. lacrymosus, P. lucipetus, P. muraschkinzewi, P. nocturnus, P. offlexorius, P. palpebrarum, P. rizalensis,* and *P. sinensis.*

SUMMARY

The development of *Philophthalmus gralli* Mathis and Leger, 1910 in Hawaii has been followed from the metacercaria to sexually-mature adult in experimental infections of chickens. Variations due to age and type of host were found to affect the reproductive organs. Species of the genus are compared in regard to some constant characteristics. *P. anatinus* Sugimoto, 1928 and *P. nyrocae* Yamaguti, 1934 are regarded as synonyms of *P. gralli.* Other species in the genus need to be examined further to determine their validity.

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Revision of the Genus *Plectus* (Nematoda: Plectidae)

A. R. Maggenti*

The genus *Plectus* was proposed by Bastian in 1865 when he described nine species from England. A type for the genus was not established until 1905 when Bastian, in a letter to Stiles and Hassall (1920), designated *Plectus parietinus* Bastian, 1865 as the type species for the genus. The family Plectidae was proposed by Örley (1880) and the subfamily Plectinae was proposed by Micoletsky (1922).

Many changes have been proposed for Bastian's species by subsequent workers. In 1933 de Coninck and Schuurmans Stekhoven made *Plectus granulosus* the type of the genus *Anaplectus*. Following Bastian, Bütschli in 1873 described seven additional species of *Plectus* and redescribed two of Bastian's 1865 species from specimens collected in Germany. *Plectus auriculatus* Bütschli, 1873 was removed by Cobb (1913) and placed in his newly-proposed genus *Wilsonema*. In 1880 de Man described five new species of *Plectus* from the Netherlands. Of these, one has been placed as a synonym of *Anaplectus granulosus* and another, *Plectus otophorus*, was transferred by Cobb (1913) to the genus *Wilsonema*. Micoletsky (1922) proposed several varieties of *Plectus cirratus* Bastian, 1865. Schneider (1939) redescribed ten species of *Plectus*, and he did not recognize the genus *Anaplectus* de Coninck and Schuurmans Stekhoven, 1933. T. Goodey (1951) placed *Anaplectus* in synonymy with *Plectus*.

Sixty-eight nominal species have been described in the genus *Plectus*. Thirteen of these nominal species have (here or previously) been placed in other genera, 18 more are placed in synonymy, 15 are designated as *species inquirendae*, and six are designated as *nomen dubium*. The latter two categories contain those nominal species with inadequate descriptions and illustrations, thereby prohibiting accurate placement. In some cases (*nomen dubium*) even generic assignment is not possible. Some of the forms in *species inquirendae* may later prove to be valid species and others will undoubtedly become synonyms of known species. The present work recognizes 16 species of *Plectus* and of these four are new.

Three genera have been recognized from species originally described in *Plectus: Wilsonema* Cobb, 1913; *Anaplectus* de Coninck and Schuurmans Stekhoven, 1933 and *Paraplectonema* Strand, 1934. Of these only *Anaplectus* has not received wide acceptance. *Anaplectus* is a valid genus and should be used as such. The genus is here removed from the family Halaphanolaimidae de Coninck and Schuurmans Stekhoven, 1933 and placed in the family Plectidae Örley, 1880. *Anaplectus submersus* (Hirschmann, 1952) n. comb. is proposed for *Plectus submersus* Hirschmann, 1952 because the illustrations and characters given by Hirschmann agree with the characters of *Anaplectus* as this genus is presently understood. A new combination is proposed (*Chronogaster multitubiferous* (Imamura, 1931) n. comb.) for *Plectus multitubiferous* Imamura, 1931.

It is also here proposed that the subgeneric name *Plectoides* be dropped from usage in the genus *Plectus*. This subgeneric division was proposed by de Man and was based upon the presence of tuberculi (denticles) in the valve of the posterior bulb. It is now known that the valve in the posterior bulb of all *Plectus* species is denticulated.

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I wish to extend sincere gratitude to Dr. M. W. Allen for his encouragement, advice and interest throughout the progress of this work and also for collecting many of the specimens studied. Appreciation is extended to Dr. J. B. Goodey, Dr. D. J. Raski, Dr. S. A. Sher, Dr. A. C. Tarjan and Mr. G. Thorne for their contributions of specimens examined.

MATERIALS AND METHODS

Holotypes and paratypes have been selected for all new species. They are mounted in dehydrated glycerine and are on file with the University of California Nematode Survey Collection, Davis.

Neotypes have been selected for four species from what I considered within a reasonable area of the type locality. Bütschli was no more specific about original localities than Germany, and de Man no more than Holland. Neotypes are mounted in dehydrated glycerine and are on file with the University of California Nematode Survey Collection, Davis, and at the Rothamsted Experiment Station, England.

The dimensions for the species are given according to the formula of de Man (1880). For this formula body width was measured just anterior to the vulva; esophageal length, from the anterior extremity of the body to the posterior end of the esophago-intestinal valve; tail length, from the anus to and including the spinneret; stoma length, from the anterior extremity to the base of the meso-metastom (the prostom includes only the expanded anterior portion of the stoma and the meso-metastom the contracted posterior portion); amphid position, from the anterior extremity of the body to the posterior edge of the external opening of the amphid; anterior and posterior ovaries, from the vulva to their most anterior or posterior extreme. The spinneret measurement includes the entire cuticularized tube and not just the external portion of the tube. In the holotype and neotype descriptions the measurements in parenthesis refer to population range.

More than 2,000 specimens of *Plectus* and *Anaplectus* have been studied. The majority of specimens were fixed in F.A.A. (formalin six parts, 95% ethyl alcohol twenty parts, glacial acetic acid one part, water forty parts) and mounted in dehydrated glycerine. Many species were studied as live mounts and also as formalin mounts. Face views, mounted in glycerine jelly, were also prepared.

SYSTEMATICS

Genus *Plectus* Bastian, 1865

SYNONYM: *Pycnolaimus* Cobb, 1920, p. 258

DIAGNOSIS: Plectinae. Cuticle marked by transverse striations. Striations interrupted by longitudinal alae. Lips, six; four cephalic setae located just posterior to lips. Stoma open, length generally twice width of lip region. Prostom expanded, meso-metastom combined, width usually one-half width of prostom. Amphids circular or broad ovals. Cervical papillae setiform, present at level of excretory pore, and situated in or near longitudinal alae. Excretory pore ventral with cuticularized excretory duct (tortuous) which leads to a ventral excretory cell. Posterior bulb with triradiate denticulated valve. Tail contains three caudal glands, which open through a cuticularized orifice (spinneret). Body with scattered setae. Hypodermal glands and orifices, present or absent. Female: vulva usually near equatorial, ovaries didelphic, amphidelphic, and reflexed. Male: testes diorchic and opposed leading into a single vas deferens. Spicules paired and asymmetrical. Guber-

naculatum present. Cuticularized preanal tubuli present or absent; ventrolmedian preanal seta present or absent; postanal setae present; postanal papillae present or absent.

KEY TO THE SPECIES OF *Plectus* BASTIAN, 1865

1. Vulva at 75% ..... *vindobonensis* von Gunhold, 1953
   Vulva at less than 75% 2
2. Cervical papillae setiform, situated at level of excretory pore and dorsal to the longitudinal alae. Wing area with one longitudinal ala ..... *annulatus*, n. sp.
   Cervical papillae setiform, situated at level of excretory pore and located between longitudinal alae. Wing area with two or more longitudinal alae 3
3. Wing area with two longitudinal alae 4
   Wing area with four longitudinal alae ..... *thornei* Rühm, 1956
4. Lip region distinctly set-off from neck 5
   Lip region not set-off from neck 6
5. Tail length less than six anal body diameters. Cheilorhabdions distinct, lip region set-off by deep constriction. Subdorsal caudal seta near tail tip on left side of body ..... *parietinus* Bastian, 1865
   Tail length more than seven anal body diameters. Cheilorhabdions not distinct, lip region set-off by shallow constriction. No subdorsal caudal seta near tail tip ..... *elongatus*, n. sp.
6. Tail conoid, length less than four anal body diameters 7
   Tail elongate, length more than four anal body diameters 8
7. Cephalic setae long (length two-thirds width of lip region), lanceolate. Small species usually less than 0.6 mm. long ..... *armatus* Bütschli, 1873
   Cephalic setae stout (length less than one-third of width of lip region). Short stout setae scattered on neck. Body length more than 0.6 mm. long ..... *assimilis* Bütschli, 1873
8. Amphids large, diameter one-fourth or more width of neck 9
   Amphid diameter less than one-fourth width of neck 11
9. Lips low, rounded, height of lips equals one-fourth width of lip region. Cephalic setae length equals one-third width of lip region. Prostom one-third length of meso-metastom ..... *varians*, n. sp.
   Height of lips equals one-half width of lip region. Cephalic setae length equals one-half width of lip region. Meso-metastom at least twice length of prostom 10
10. Tail more than 7 (seven) anal body diameters ..... *inquirendis* Andrássy, 1958
    Tail less than seven anal body diameters ..... *rhizophilus* de Man, 1880
11. Body length less than 0.9 mm. 12
    Body length more than 0.9 mm. 15
12. Tail length less than seven anal body diameters 13
    Tail greatly elongated, length greater than seven anal body diameters, tail ventrally curved in distal one-third ..... *longicaudatus* Bütschli, 1873
13. Stoma almost cylindroid, prostom only slightly expanded 14
    Stoma normal, prostom wider than meso-metastom. Prostom two-thirds length meso-metastom. Lip height one-half width of lip region ..... *acuminatus* Bastian, 1865

14. Prostom one-third length of meso-metastom. Cephalic setae one-half as long as width of lip region. Lips conoid-connate ..... *parvus* Bastian, 1865
    Prostom obscure. Cephalic setae as long as width of lip region. Lips low, rounded and obscure ..... *cornus*, n. sp.
15. Tail length less than six anal body diameters, esophagus less than seven times width of neck at bulb, esophageal intestinal valve one-third body width at its level ..... *cirratus* Bastian, 1865
    Tail length more than six anal body diameters, esophagus length more than seven times width of neck at bulb, esophageal intestinal valve one-half width of neck at its level ..... *palustris* de Man, 1880

Plectus parietinus Bastian, 1865 (Fig. 1, 2 A-E)

SYNONYMS: *Plectus fusiformis* Bastian, 1865, p. 121; *Plectus velox* Bastian, 1865, p. 119; *Plectus ornatus* Bütschli, 1873, pp. 91-92; *Plectus intermedius* Cobb, 1893b, p. 827; *Plectus patagonicus* de Man, 1904a, pp. 41-45; *Plectus hawaiiensis* Cobb, 1906, pp. 184-185. *Plectus antarcticus* de Man, 1904b, pp. 8-10; *Plectus naticochensis* Steiner, 1920, p. 13; *Plectus pusteri* Fuchs, 1930, pp. 530-533.

DIMENSIONS: 29 Females—L = .932-1.7 mm., a = 12.8-25.5, b = 3.2-5.2, c = 8.0-15.5, v = $^{9.5\text{-}16.4}45\text{–}52^{11.5\text{-}16.5}$, Stoma = 20–34 microns; 2 Males—L = 1.2-1.3 mm., a = 18.4-22, b = 4.3-4.8, c = 12-13.2, T = 30-35%.

FEMALE (NEOTYPE): L = 1.38 mm., a = 20.4, b = 4.3, c = 11.6, v= $^{15}50^{17.2}$, Stoma = 26.5 microns. Body plump, spindle shaped, tapering more posteriorly than anteriorly. Cuticle distinctly marked by transverse striae, in some specimens striations continue to tail tip. Striations interrupted on both sides of body by two longitudinal alae, wing area occupies one-eighth of body diameter at vulva. Hypodermal glands prominent from cervical region to tail. Lip region rounded, one-third as high as broad. Lips conoid and distinct to and at their base. Lips well set off from neck by constriction. Four cephalic setae, large and well developed, 5 microns (3-5 microns), located two annules posterior to lips. Stoma length 26.5 microns (20-30 microns), one-third longer than width of lip region. Cheilostom distinct. Prostom two-thirds length of meso-metastom. Amphid diameter 2.5 microns (2-3.3 microns) one-sixth body diameter at their level. Esophagus approximately one-fourth of body length. Excretory pore 55.5% (49-60%) of esophagus length. Nerve ring about one-half body diameter anterior to excretory pore. Cervical papillae setiform, between longitudinal alae and located at level of excretory pore. Esophago-intestinal valve one-fifth of body diameter at its level. Vulva usually equatorial, 50% (45-52%) of body length. Vagina extends into body one-fourth of body diameter at vulva. Eggs 48 microns × 62 microns (35-48 microns × 52-70 microns). Rectum 35.5 microns (23-36 microns) long. Tail length 3.3 (3-4) anal body diameters, uniformly conoid, ventrally arcuate, terminus blunt. Cuticularized orifice of caudal glands (spinneret) 5 microns (3-5 microns). Sub-dorsal distal caudal seta located slightly more than spinneret length back from tail tip and on left side.

MALE: Male similar to female. Testes diorchic, opposed, occupy 3035% of body. Spicules asymmetrical in size and/or shape. Two males available for examination, one specimen, right spicule 64 microns, left spicule 56 microns; second male right spicule 44 microns and left spicule 60 microns.

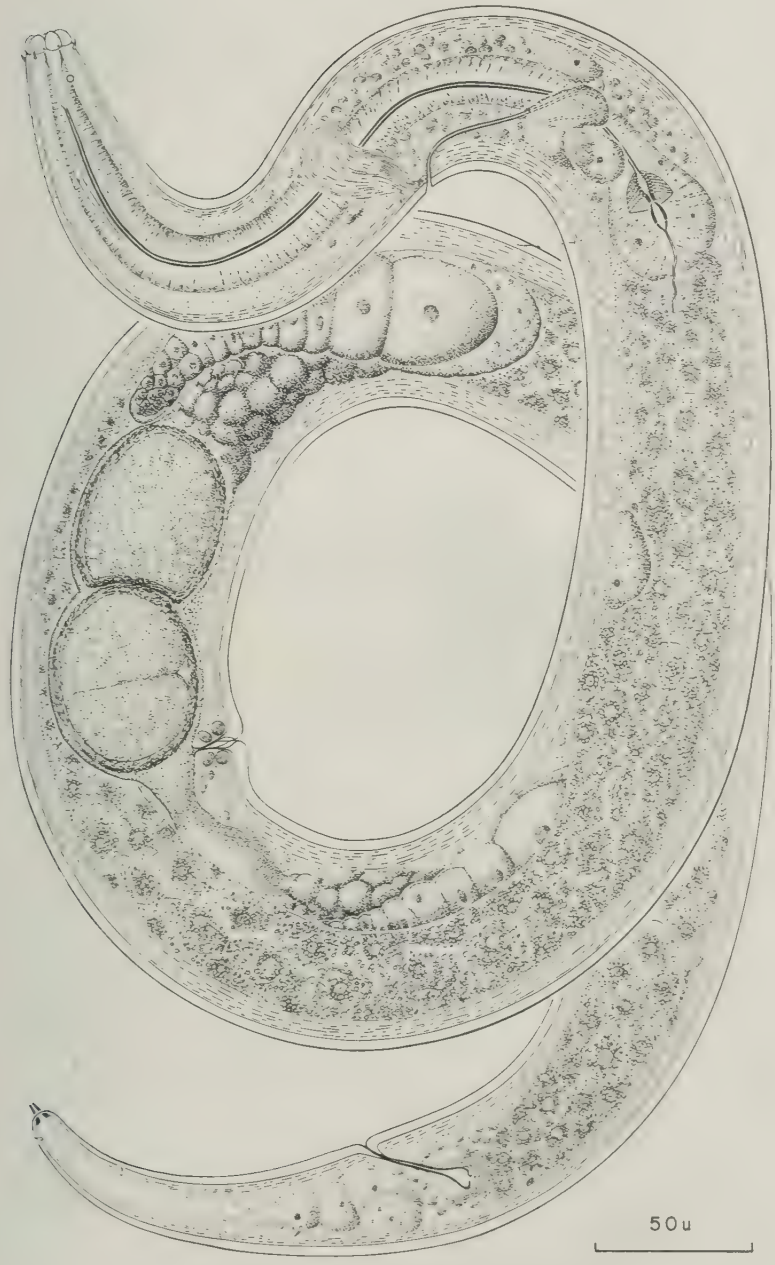

Fig. 1. *Plectus parietinus.* Female.

Both specimens had five well-developed preanal supplementary organs, which extended into body 24-26 microns. Gubernaculum: dorsal process 10.6 microns, ventral arm 12.8 microns. Preanal ventral seta present. Seven pairs of caudal setae, three subventral and four subdorsal. Male also has two caudal papillae located on posterior one-third of tail.

Neotype: Female collected December 11, 1959 by J. B. Goodey, Catalogue no. 90/1/2. Rothamsted Experimental Station, Harpenden, Herts, England.

Habitat: *Sagina* spp. (Pearlwort).

Neotype locality: Broadmoor, Berks, England.

Plectus parietinus is distinguished from all other species of *Plectus* by the prominent hypodermal glands, the relatively small amphid, the distinct and well set-off lips and by the subdorsal distal caudal seta on the left side of the tail.

Plectus fusiformis, P. velox, P. ornatus, P. intermedius, P. patagonicus, P. naticochensis and *P. pusteri* are placed in synonmy with *P. parietinus* because the original descriptions and illustrations show no contradictory characters to the description given here or to those characters set forth by Bastian (1865).

Plectus hawaiiensis and *P. antarcticus* are placed in synonomy because specimens examined from Hawaii and the Antarctic; agreeing with the description given by Cobb and de Man respectively are *P. parietinus*.

Specimens of *Plectus parietinus* were examined from: the United States, many localities in California, Colorado, Connecticut, Hawaii, Idaho, Kansas, Kentucky, New Jersey, Nevada, Wisconsin; from other areas of the world: Antarctic, Australia, Canada, England, Ireland, and many localities in the Netherlands.

Plectus vindobonensis von Gunhold, 1953 (Fig. 3, D-E)

Dimensions: 2 Females—520-526 microns. a = 22.2-22.4, b = 5.5-5.7, c = 7.2-7.4, V = 75, Stoma = 12.8-13 microns.

Anterior end slightly narrowed, bears six clear slightly offset lips. Head setae normally arranged. Stoma narrow, running straight back. Amphids large, spiral (?) and characteristic and lie at level of first one-fourth of buccal cavity. Fore-end appears similar to *Paraplectonema pedunculatus* (Hofmänner, 1913) Strand, 1934 in relation to height of setae. Esophagus relatively long, supplied with median swelling and ends with simple, valveless (?) weak pear-shaped bulb. Vulva located far posteriorly, a characteristic which separates this species immediately from the remaining representatives of the genus. Gonads characteristic of *Plectus*. Tail relatively long, towards back symmetrically tapered and ends with offset spinneret. Cuticle weakly ringed, provided dorsally and ventrally with difficult-to-see setiform papillae. Exact position of species not easily determined. (Description and measurements from von Gunhold, 1953.)

Species easily distinguished from remaining species of genus by posteriorly-located vulva.

Habitat: Foundry mound near Vienna, in forest litter under *Quercus cerris*.

Plectus annulatus, n. sp. (Fig. 4, A-D)

Dimensions: 9 Females—L = .948-1.13 mm., a = 22.4-26.7, b = 3.58-4.24, c = 9.1-10.2, V = $^{9.1\text{-}12.6}50\text{-}55^{9.4\text{-}11}$, Stoma = 22-26 microns.

Female (holotype): L=1.03 mm, a=22.4, b=3.83, c=9.6, V=$^{10.2}50^{9.5}$ microns. Body slender, tapered little anteriorly, more posteriorly. Cuticle

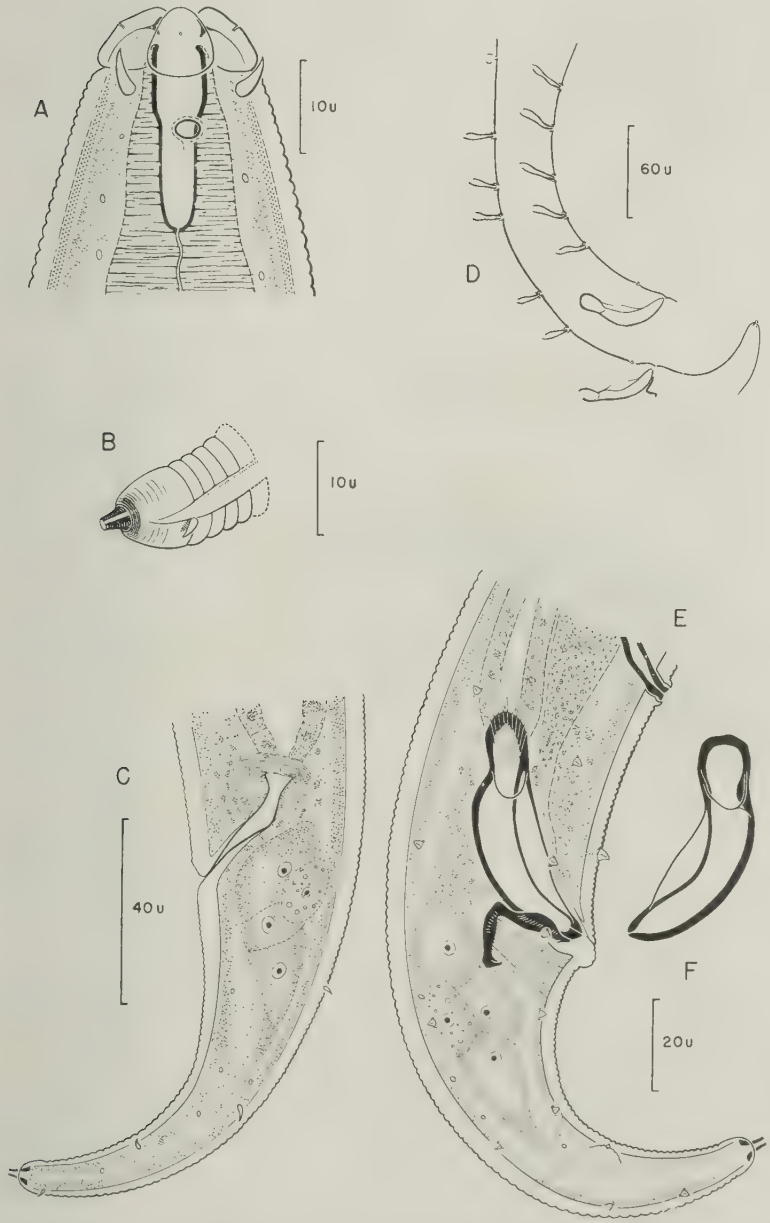

Fig. 2. *Plectus parietinus*. A—Head. B—Spinneret. C—Female tail. D— Preanal tubuli male tail. E—Male tail. F—Left spicule.

thick, marked by deep, distinct transverse striae. Striae interrupted on each side of body by longitudinal alae. Each wing area composed of a single longitudinal ala, flattened on top and with only the slightest hint of a central depression. Lip region rounded about half as high as width. Six lips bluntly conoid, each distinct at base. At oral opening each lip extends to a short point. Lip region set off by deep constriction. Four well-developed cephalic setae 4.5 microns (4-5 microns) long, located two to three annules posterior to lip region. Stoma length 26 microns (22-26 microns) slightly more than twice width of lip region. Cheilostom well developed, plates distinct. Prostom one-third length of meso-metastom. Amphids 18.5 microns (16-19 microns) from anterior extremity located at level of meso-metastom. Amphid diameter 3 microns (3-3.3 microns) one-fifth width of neck at their level. Esophagus approximately one-fourth of total body length. Excretory pore located at 56.5% (54-58%) of esophagus length. Nerve ring just anterior to excretory pore. Cervical papillae setiform, situated outside and dorsal to longitudinal alae, just posterior to excretory pore. Length of esophagointestinal valve slightly more than one-third body width at its level. Vulva usually equatorially located, 50% (50-55%) of total body length. Vagina extends slightly less than one-half body diameter into body at vulva. Rectum 27.5 microns (25.5-33 microns). Tail 4.7 (3-5) anal body diameters. Cuticularized orifice of caudal glands (spinneret) 3.5 microns (3.3-4 microns).

Male: Not known.

Holotype: Female collected August 19, 1957 by A. R. Maggenti, catalogue no. 120, University of California Nematode Survey Collection.

Paratypes: Twelve females same data as Holotype.

Type Habitat: Moss, Oregon Ash (*Fraxinus oregona*).

Type locality: South fork of Tuolumne River, Lumsden Bridge, United States Forest Service Camp Grounds, Stanislaus National Forest, Tuolumne County, California, U.S.A.

Plectus annulatus n. sp. differs from all other known species of *Plectus* by the well-developed cheilostom, the one longitudinal ala on each side of the body, and the placement of the cervical papillae dorsal to the longitudinal alae.

Specimens of this species were examined from the following additional habitats and localities in California: tree moss, Hecker Pass, Santa Clara County; moss from California laurel, near a small stream, Inverness, Marin County.

Plectus thornei Rühm, 1956 (Fig. 5, A-G)

Dimensions: Female—L = 0.87-1.065 mm., a = 22.59-25.35, b = 4.07-4.53, c = 8.28-14.44, V = 48.08-50, Stoma = .032 mm. Male—L = 0.825-0.93 mm., a = 23.57-26.57, b = 4.13-4.22, c = 11.46-12.65, Stoma = .032 mm., Spicules 0.032-0.036 mm., gubernaculum 0.009-0.011 mm.

Female: Cuticle thick and clearly annulated. Longitudinal stripes appear with fine knot-like thickenings (it appears, from Rühm's illustrations that four longitudinal alae are present in each wing area). Scattered setae on body of adults and larvae. Six distinct lips, each exhibits two granular structures. Four thin cephalic setae, two ventral submedian and two dorsal submedian. Amphids clearly visible. Prorhabdions strongly cuticularized, slightly curved and non-articulate. Esophagus begins on posterior portion of prostom. Meso-metasom funnel-shaped, uniformly cuticularized. Esopha-

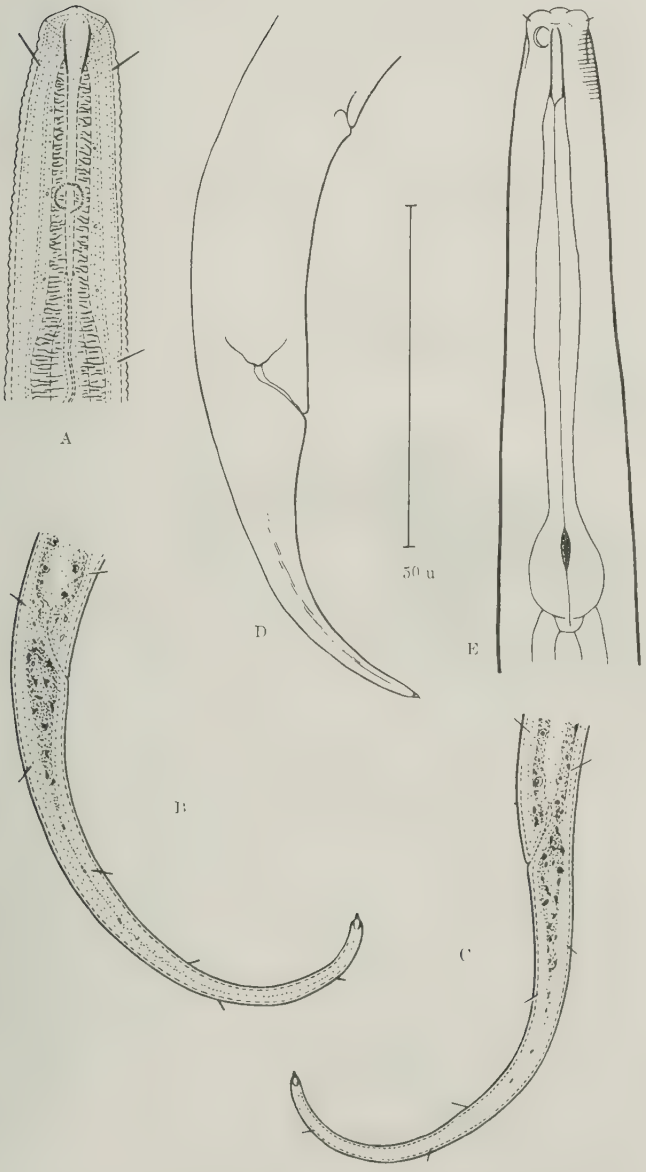

Fig. 3. A-C—*Plectus inquirendis*. A—Head. B—Female tail. C—Female tail (after Andrássy, 1958). D-E—*Plectus vindobonensis*. D—Female tail. E—Head (after von Gunhold, 1953).

gus, at first, slender, then widens regularly to isthmus; posterior bulb oval. Triradiate denticulated valve of posterior bulb well developed. Nerve ring at junction of corpus and isthmus. Vulva equatorial. Gonads paired, short with swollen club-like ovaries. Tail slender, terminates with a spinneret. (Descriptions and measurements after Rűhm 1956.)

Male: Similar to female. Two preanal supplementary tubuli consistently present. Two postanal papillae. Spicules paired, gubernaculum well developed. Tail terminates with spinneret.

Habitat: Spruce mold in larval galleries of *Ips typographus*.

Locality: Regensburg, Germany.

This species may be distinguished from other species of *Plectus* by the four longitudinal alae in each wing area. Rűhm felt that this species should be placed close to *Anaplectus granulosus* (used as *Plectus granulosus* in Rűhm's paper). Rühm's illustrations, however, indicate that this is a species of *Plectus* and not *Anaplectus*.

According to Rűhm, large numbers of males were collected in mid-September from decomposed spruce mold. The proportion of females to males was 5:1.

Plectus elongatus, n. sp. (Fig. 4, E-G)

Dimensions: 9 Females—L = .712-1.03 mm., a = 18.7-25.4, b = 4.03-4.96, c = 4.5-5.6, V = $^{9\text{-}15.5}43\text{-}48.5^{10\text{-}15.5}$, Stoma = 19.3-24.3 microns.

Female (holotype): L = .712 mm., a = 25.4, b = 4.2, c = 4.56, V = $^{9.1}43.5^{9.1}$, Stoma = 19 microns. Body long and rather slender. Anterior tapering most pronounced from just posterior to stoma. Posterior tapers to long slender tail. Cuticle faintly marked by transverse striae, most pronounced on cephalic region and tail. Transverse striae interrupted on both sides of body by two longitudinal alae, each wing area occupies approximately one-eighth of body diameter at vulva. Lip region with six conoid lips, distinct to and at base, lip region set off from neck by constriction. Four cephalic setae 2.5 microns (2-3.6 microns), located two annules posterior to lip region. Stoma length 19 microns (19-24.3 microns), slightly longer than twice width of lip region. Cheilostom indistinct, prostom one-third of meso-metastom length. Amphids 11.5 microns (11-14.5 microns) from anterior extremity situated at level of meso-metastom. Amphid diameter 2.5 microns (2.3-3 microns), approximately one-fourth to one-fifth of body width. Excretory pore 55.5% (51.5-61%) of esophagus length. Nerve ring just anterior to excretory pore. Cervical papillae setiform situated between longitudinal alae and just posterior to excretory pore. Esophagointestinal valve one-third body width at its level. Vulva anteriorly located 43.5% (43-49%) of body length. Vagina extends into body one-third of vulval body diameter. Eggs 28-34 microns × 56 microns. Rectum length 22 microns (17.5-26 microns). Tail long and slender, length about nine anal body diameters, distal three-fourths almost uniform diameter to tip. Tail usually ventrally curved at least distal fourth. Cuticularized orifice of caudal glands (spinneret) 2.5 microns (2-3 microns).

Male: Not known.

Holotype: Female collected October 2, 1957 by A. R. Maggenti, catalogue no. 118, University of California Nematode Survey Collection.

Paratypes: Six females same data as holotype; four females collected February 18, 1945 by J. McCarty, Hopland, California.

Type habitat: Moss along stream.

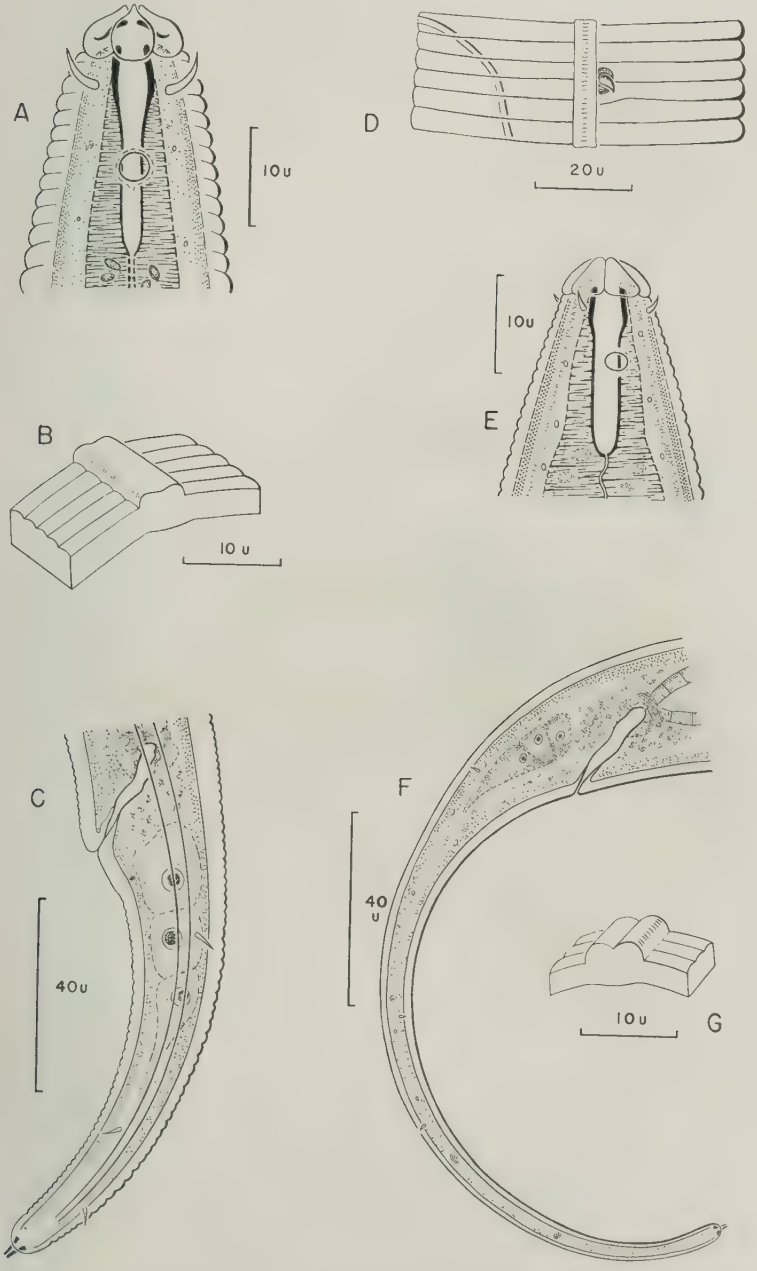

Fig. 4. A-D—*Plectus annulatus*. A—Head. B—Longitudinal ala. C—Female tail. D—Cervical papilla. E-G—*Plectus elongatus*. E—Head. F—Female tail. G—Longitudinal alae.

TYPE LOCALITY: South Fork of Strawberry Creek, University of California Campus, Berkeley, California, U.S.A.

Plectus elongatus is distinguished from other species of *Plectus* by the set-off lip region and by the long slender tail.

Specimens of this species were examined from the following additional habitats and localities in California, U.S.A.: soil around oak association, six miles east of Hopland, Mendocino County; stream side, near Shell Beach, Inverness, Marin County; north of Marsh Creek, six miles southwest of Brentwood, Contra Costa County; moss, Tilden Regional Park, Contra Costa County; soil, guayule nursery, Alisal. In Ireland: soil from beet field Ballybunion.

Plectus armatus Bütschli, 1873 (Fig. 6, A-C)

DIMENSIONS: 8 Females—L = 0.34-0.46 mm., a = 13.5-16, b = 3.5-4.2, c = 7.7-10.5, V = $^{7.3\text{-}14.2}47\text{-}51^{9.3\text{-}12}$, Stoma = 14-18 microns.

FEMALE: Body tapers to both extremities, especially posteriorly. Cuticle marked by transverse striations, beginning at cephalic setae. Striations fine but pronounced on cephalic region and tail. Transverse striations interrupted on both sides of body by two longitudinal alae. Each wing area occupies one-seventh of body width at vulva. Lip region truncate, with six low, rounded, almost obscure lips. Lips not set off by a marked constriction. Four cephalic setae at base of lips. Setae length equals two-thirds width of lip region. Cephalic setae broad and flattened at base, taper rapidly to apex in distal two-thirds. Cheilostom obscure. Stoma twice as long as width of lip region (15-18 microns). Prostom one-third length of meso-metastom. Amphids usually located at middle of stoma. Amphid diameter one-third to one-fourth width of neck at their level. Esophagus approximately one-fourth body length (b = 3.5-4.2). Excretory pore ventral, located two-thirds of esophageal length from anterior extremity. Ventral seta 3-5 annules posterior to excretory pore. Nerve ring just anterior to excretory pore. Cervical papillae setiform and situated between longitudinal alae just posterior to level of excretory pore. Esophago-intestinal valve short, one-sixth body width at its level. Vulva pre- to post-equatorial in position, 47-51% of body length. Vagina extends into body one-sixth of body width. Eggs 20-23 microns × 26-50 microns. Tail narrow, tapers rapidly posterior to anus, length approximately four anal body diameters. Cuticularized orifice of caudal glands (spinneret) 1.5 to 2.0 microns long. Two subventral rows of setae along entire body.

MALE: Not known.

TYPE HABITAT: At the roots of wild (wood) strawberry (*Fragaria vesca*).

TYPE LOCALITY: Germany.

Plectus armatus is associated in the key with *P. assimilis* Bütschli, but can be readily separated by the large, basally flattened cephalic setae and the subventral rows of setae, which extend the length of the body. They both differ from other species of *Plectus* by their short tail and body shape.

This species was originally described by Bütschli from Germany. In his original description Bütschli described six cephalic setae; however six setae were not seen on any specimens examined in this study.

Specimens of this species were examined from the following habitats and localities in California: soil at base of oak trees, Strawberry Canyon, Berkeley; soil, grassy slope, University of California Campus, Berkeley; soil about lupine and wild strawberries, stream bank moss, Berkeley.

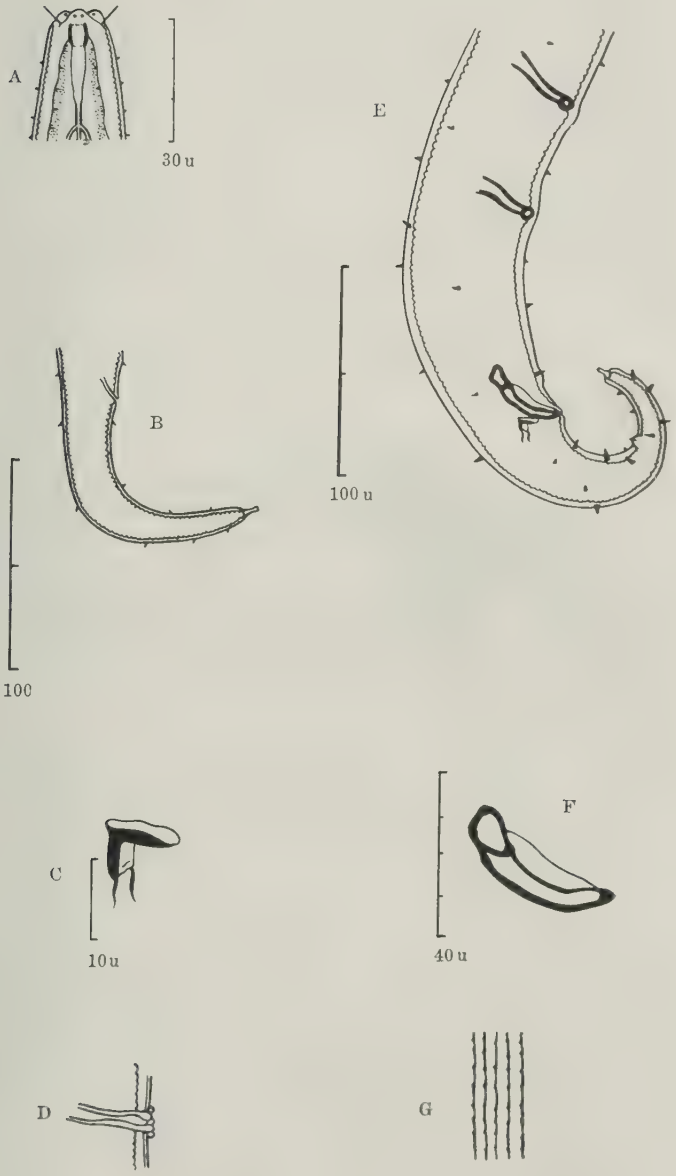

Fig. 5. *Plectus thornei*. A—Head. B—Female tail. C—Gubernaculum. D—Preanal tubulus male. E—Male tail. F—Spicule. G—Longitudinal alae (after Rühm, 1956).

Plectus assimilis Bütschli, 1873 (Fig. 6, D and E)

Dimensions: 10 Females—L = .72-.84 mm., a = 14-17.2, b = 4-5, c = 10.4-13.4, V = $^{9.2\text{-}15.5}47\text{-}51^{12.8\text{-}17.1}$, Stoma = 20-27 microns.

Female: Body stout, tapering only slightly anteriorly; posteriorly body tapers rapidly to short tail. Cuticle marked by transverse striae most prominent in cephalic region. Transverse striae interrupted on each side of body by two longitudinal alae, each wing area occupies approximately one-ninth of body width at vulva. Lip region truncate-rounded, very low, less than one-fourth as high as broad. Six very low, connate lips, not set off from neck. Lips delineated by beginning of transverse striations. Four cephalic setae, broad at base, tapering rapidly (4-4.6 microns). Cephalic setae located one or two annules behind lips. Stoma length (20-26.6 microns) equal to one and one-half times lip region width. Stoma wide, prostom usually one-third width of neck at its level. Cheilostom indistinct, prostom length about one-half length of meso-metastom. Amphids 6.6-10 microns from anterior extremity located at level of prostom. Amphid diameter (2-2.6 microns) one-fifth of neck width at their level. Esophagus approximately one-fourth of total body length. Excretory pore located at 52-60% of esophagus length. Nerve ring just anterior to excretory pore, cervical papillae setiform, situated between the longitudinal ridges just posterior to excretory pore. Esophago-intestinal valve length slightly more than one-fourth body width at its level. Vulva equatorial 47-51% of total body length, vulva lips not protruding. Vagina extends into body about one-fifth of body diameter at vulva. Eggs 70 microns × 32-34 microns. Rectum length 24-28 microns. Tail short (c = 10.4 to 13.4) tapering rapidly to very narrow tip. Tail length three to three and one-half anal body diameters. Five caudal setae: two subdorsal and three subventral. Body with stout setae irregularly placed but concentrated in cephalic region. Cuticularized orifice of caudal glands (spinneret) 2-2.6 microns in length.

Male: Not known.

Type habitat: Moss on tree trunk.

Type locality: Germany.

P. assimilis can be separated from *P. armatus* by the shape of the cephalic setae and the stout setae scattered over the body. In comparison with other *Plectus* the shape of the body (Rhabditis-like), the shape of the lip region, and the anteriorly placed amphids are considered diagnostic.

Although scattered setae are a universal occurrence in the genus *Plectus,* it is their large stout form that is different in *Plectus assimilis,* and their concentration on the neck region.

Specimens of this species were examined from the following localities in the United States: California, Utah and Washington.

Plectus varians, n. sp. (Fig. 7, A-D)

Dimensions: 10 Females—L = .667-.864, a = 21.1-28, b = 3.4-4.5, c = 6.7-9.2 V = $^{10.8\text{-}11.2}48\text{-}51^{8.5\text{-}12.5}$, Stoma = 19-23.3 microns. 1 Male—L = .92 mm., a = 25.4, b = 4.3, c = 9.35, T = 54.5, Stoma = 22.6 microns. Right spicule = 32 microns. Left spicule = 26 microns, gubernaculum = 11 microns.

Female (holotype): L = .687 a = 25.4, b = 3.67, c = 7.31, V = $^{11}50^{9.75}$, Stoma = 19.5 microns. Body slender, tapered to both extremities, more pronounced posteriorly. Cuticle marked by faint transverse striations.

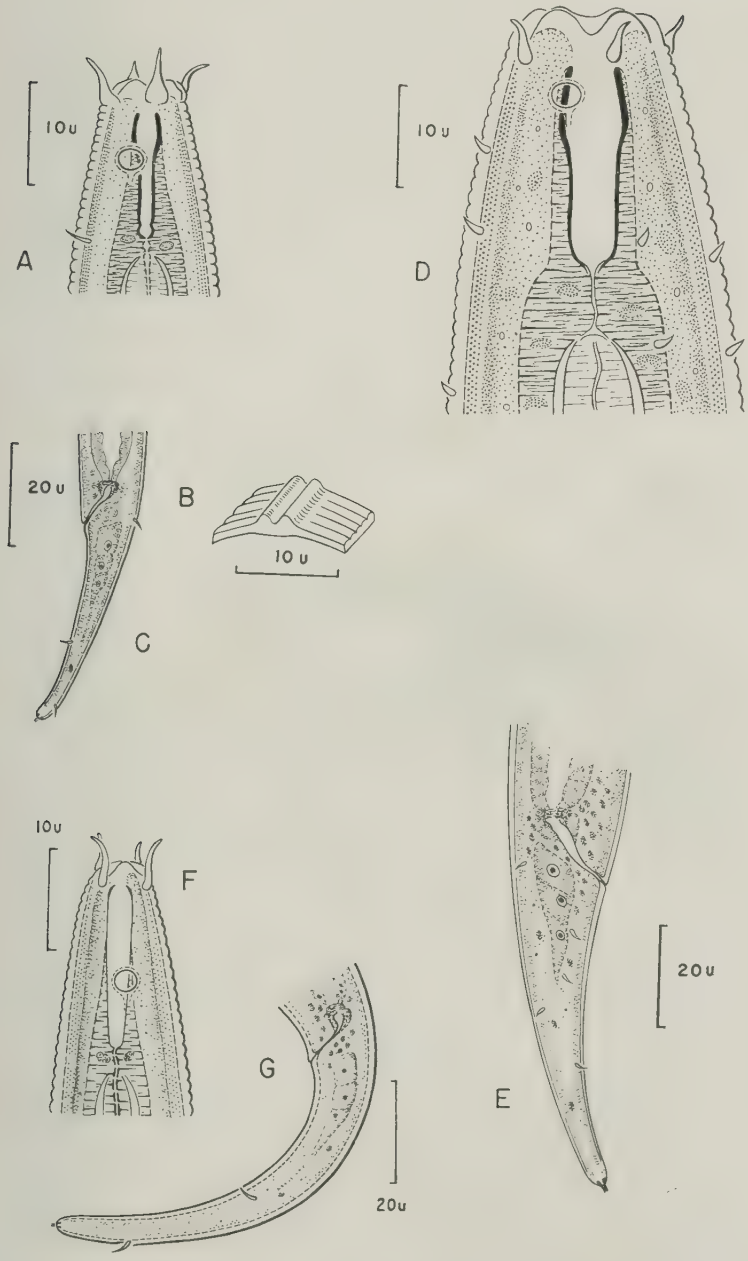

Fig. 6. A-C—*Plectus armatus*. A—Head. B—Longitudinal alae. C—Female tail. D-E—*Plectus assimilis*. D—Head. E—Female tail. F-G—*Plectus cornus*. F—Head. G—Female tail.

Striations interrupted by longitudinal alae, each wing area contains two longitudinal alae and occupies one-seventh body diameter at vulva. Height of lips slightly more than one-fourth width of lip region. Lip region delineated by beginning of transverse striations. Four slender cephalic setae 3.5 microns (2.6-3.5 microns), located two annules posterior to lip region. Stoma 19.5 microns (19-23.3 microns), length twice width of lip region. Cheilostom obscure. Prostom one-third length of meso-metastom. Amphids located near level of posterior half of stoma 15.5 microns from anterior extremity. Amphid diameter 3 microns (2.6-3.3 microns) occupying one-third width of neck. Esophagus about one-fourth of body length. Excretory pore slightly posterior to middle of esophagus 57.5% (51-59.5%). Nerve ring just anterior to excretory pore. Cervical papillae setiform, situated between longitudinal ridges slightly posterior to level of excretory pore. Esophagointestinal valve length, one-half width of body at its level. Vulva equatorial 50% (48-51%). Vagina extends into body one-third body diameter at vulva. Eggs 26 microns × 54 microns. Rectum length 14.5 microns (14-23 microns). Tail length about seven (5 to 7.5) anal body diameters, tapering most rapidly in proximal one-fourth, then tapering gradually to tip. Cuticularized orifice of caudal glands (spinneret) 2.6 microns (2.6-3 microns) in length.

MALE: Male similar to female; however, total length greater and excretory pore more posteriorly placed: 72% of esophagus length. Testes diorchic and opposed, occupying 54.5% of body length. Spicules asymmetrical, right spicule 32 microns; left, 26 microns long. Gubernaculum 11 microns long with prominent or conspicuous process extending anteriorly between spicules. In addition the gubernaculum has two oblique lateral processes extending beyond dorsal process. One weakly developed preanal, supplementary, noncuticularized tubulus, and one preanal seta. Tail with eight pairs of irregularly placed caudal setae, and one ventral caudal papilla.

HOLOTYPE: Female collected January 1, 1949 by H. J. Jensen, catalogue no. 119, University of California Nematode Survey Collection.

PARATYPES: One female same data as holotype; three females collected January 8, 1948 by H. J. Jensen, Mt. Vernon, Washington.

TYPE HABITAT: Moss taken from a second growth wooded area.

TYPE LOCALITY: One mile south of Summit Park, Skagit County, Washington.

Plectus varians may be readily recognized by the low, rounded lip region, the relatively large amphid, and the length of the tail.

Specimens of this species were examined from the following habitats and localities in the United States: light sandy-loam soil, Swinnimoush Slough Bridge, Skagit County, Washington; dark peat soil, four miles south of Mt. Vernon, Skagit County, Washington; moss taken from a second growth wooded area, soil shallow and rocky, one mile south of Summit Park, Skagit County Washington; soil, Oregon State College Campus, Corvallis, Oregon; soil around alfalfa, Experiment Station Farm, Reno, Nevada; soil from corn field, Kennet Square, Pennsylvania.

Plectus rhizophilus de Man, 1880 (Fig. 7, E and F)

DIMENSIONS: 10 Females—L = .67-.88 mm., a = 16.7-27, b = 4.0-4.8, c = 6-8.8, V = $^{7.6\text{-}14}42.7\text{-}49^{9\text{-}15}$, Stoma = 18-22 microns.

FEMALE (NEOTYPE): L = .71 mm., a = 23.6, b = 4.2, c = 6.56, V = $^{10.2}48.3^{9.3}$, Stoma = 21.3 microns. Body slightly tapered anteriorly, tapered strongly to posterior. Anterior tapering pronounced from base of stoma to

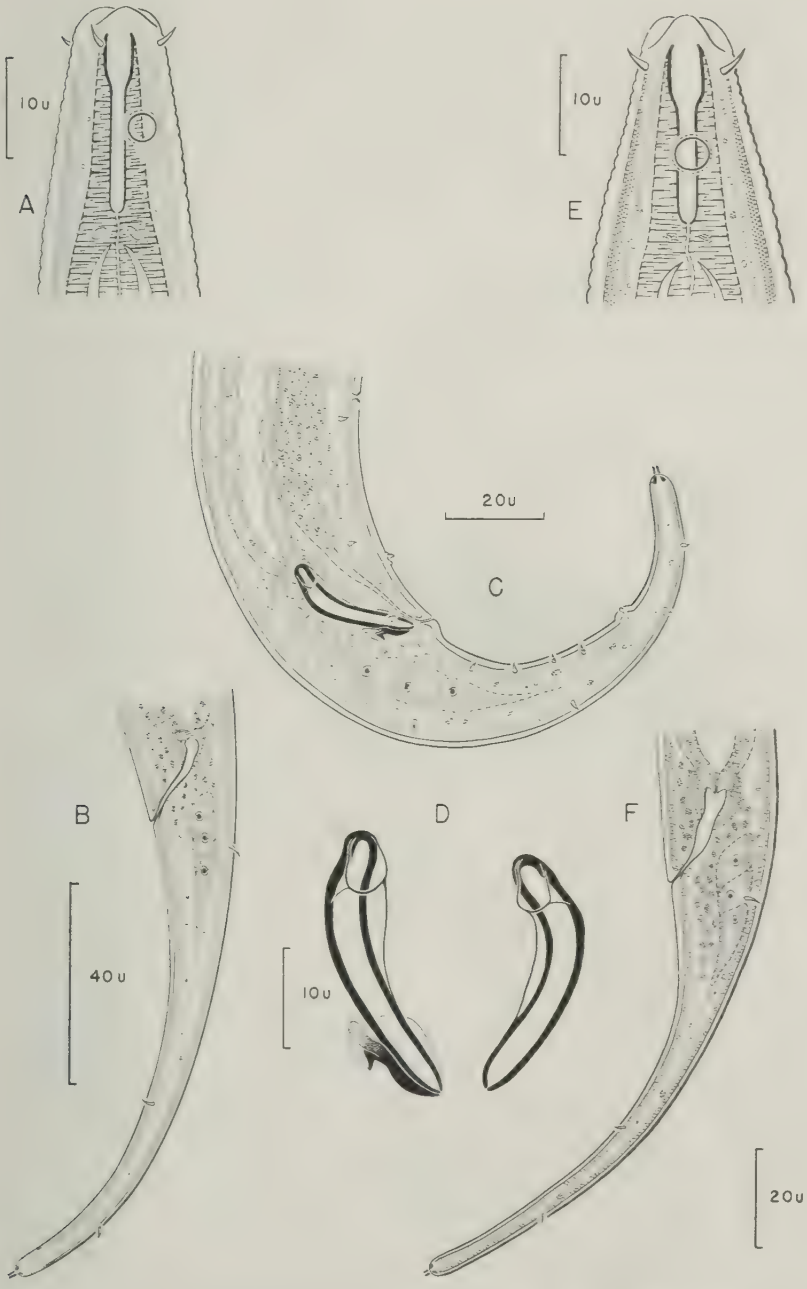

Fig. 7. A-D—*Plectus varians*. A—Head. B—Female tail. C—Male tail. D—Spicules. E-F—*Plectus rhizophilus*. E—Head. F—Female tail.

anterior extremity. Lip region one-half width of neck at base of stoma. Cuticle marked by transverse striae, more pronounced on cephalic region and tail. Transverse striae interrupted on each side of body by two longitudinal alae. Wing area occupies one-sixth of body diameter at vulva. Lips one-half as high as width of lip region. Six conoid-connate lips not well set off from neck, lips delineated from body by transverse striae. Four slender cephalic setae 3.5 microns (3-3.6 microns) length equal to one-half width of lip region, setae located two to three annules posterior to lips. Stoma length from anterior extremity 21.3 microns (18-22 microns), approximately twice width of lip region. Amphid diameter 3.5 microns (3-4 microns), one-third to one-fourth width of neck at their level. Amphids 13.5 microns (12-15 microns) from anterior extremity located at region of mesometastom. Esophagus one-fourth to one-fifth of body length. Excretory pore 52% (50-56%) of esophagus length. Nerve ring just anterior to excretory pore. Esophago-intestinal valve length slightly less than one-half body diameter at its level. Vulva usually anteriorly placed 48.3% (43-49%) of body length, vulva lips not protruding. Vagina extends into body approximately one-third of body diameter at vulva. Rectum 22 microns (16.6-22 microns). Tail length about six and one-half anal body diameters, tail tapers rapidly posterior to anus, distal two-thirds only slightly tapered. Proximal one-third of tail one-third of anal body diameter, at tail tip diameter one-sixth of anal body diameter. Cuticularized orifice of caudal glands (spinneret) 3 microns (2.6-3 microns).

Male: Not known.

Neotype: Female collected 1952 by J. W. Seinhorst, catalogue no. 122, University of California Nematode Survey Collection.

Type habitat: Soil.

Type locality: Megchelen near Gendringen, the Netherlands.

Plectus rhizophilus is distinguished from all other species of *Plectus* by the large relative size of the amphids and by the shape and length of the tail. It is separated from *Plectus varians* by the higher lip region and longer cephalic setae.

Specimens of this species were examined from: the United States, California, Colorado, and Kentucky; and from several localities in the Netherlands.

Plectus inquirendus Andrássy, 1958 (Fig. 3, A-C)

Dimensions: Female—L = 0.624-0.803 mm., a = 33.3-34.3, b = 3.3-3.8, c = 5.5-6.3, V = 47.2-49.0%.

Slender, long-tailed species. Cuticle finely, however, clearly ringed, annules 0.9 microns wide. Submedian setae present. Lateral field indistinct. Head not annulated, not offset. Lips small, papillae very small. Four cephalic setae, 3-4 microns long. Stoma very long and slender, only at anterior end a little bit wider (also chitinization stronger) its length about 3½—4 times as long as head width. Amphids a little behind middle of stoma, round, bracelet-shaped, and rather large. Esophagus in forepart somewhat thickened, isthmus slender, posterior bulb oval with relatively weak valve. Esophago-intestinal valve strongly developed and composed of two parts: forepart muscular and posterior part glandular; both parts of equal length. Intestinal lumen wide, intestinal cells with large nuclei. Rectum one-half times as long as anal body diameter, proximally bulb shaped. Vulval lips somewhat prominent, not chitinized, vagina thin walled, approximately one-third of body

diameter. Ovaries paired, reflexed, about 2½—3 times as large as corresponding body width, and asymmetrical. Sperm not found in uterus. Tail long and very slender, slowly attenuated, almost cylindrical, 10-12 times as long as anal body diameter. Spinneret small. Male unknown. (Description and measurements after Andrássy, 1958.)

TYPE SAMPLE: 2 females.

HABITAT: Detritus near a stream, Sphagnum-bog (two samples at 2300 meters above sea level). Bulgaria.

Plectus longicaudatus Bütschli, 1873 (Fig. 8, A-B)

SYNONYMS: *Plectus meridionalis* Steiner, 1916, p. 329.

Plectus kenyanus Allgén, 1952, Ark. Zool., pp. 152-154.

DIMENSIONS: 10 Females—L = .45-.65 mm., a = 22-30, b = 3.6-4, c = 4.7-7.1, V = $^{8.1\text{-}12.5}44\text{-}50^{7.5\text{-}11.6}$, Stoma = 15-20 microns.

FEMALE (NEOTYPE): L = .61 mm., a = 29, b = 3.8, c = 6.2, V = $^{9.4}46.6^{9.2}$, Stoma = 18.3 microns. Body almost cylindrical to anus, anteriorly tapers slightly from base of stoma, lip region one-half width of neck at base of stoma. Posterior extremity tapers rapidly posterior to anus to long thin tail. Cuticle marked by fine transverse striae. Transverse striae interrupted on each side of body by two longitudinal alae, each wing area occupies approximately one-sixth of mid-body diameter. Six conoid-connate lips, not distinctly set off from body. Lip height one-half width of lip region. Four slender, anteriorly directed cephalic setae, two to three annules posterior to lip region. Length of cephalic setae 4 microns (4-5 microns), approximately one-half of lip region width. Stoma length 18.3 microns (15-20 microns), slightly more than twice width of lip region. Cheilostom very indistinct. Prostom one-third of meso-metastom length and slightly more expanded, sometimes stoma almost cylindrical. Amphids located 10.5 microns (8.6-12.6 microns) from anterior extremity, situated at level of mid-stoma, diameter of amphids equals one-fifth of neck width at their level. Esophagus approximately one-fourth of the body length. Excretory pore, from anterior extremity 55.5% (52-63%) of esophagus length. Nerve ring just anterior to excretory pore. Cervical papillae setiform, located between longitudinal alae approximately one-half body width posterior to excretory pore. Length of esophago-intestinal valve one-half body width at its level. Vulva usually slightly pre-equatorial, 46.6% (44-50%) of body length, vulva lips slightly protruding. Eggs 19-23 microns × 49-52 microns. Vagina length one-third of body diameter at vulva. Rectum 17.5 microns (15-20 microns) long. Tail length approximately eight anal body diameters. Tail almost cylindrical for distal two-thirds of its length. Tail diameter for proximal one-third is one-half anal body diameter; from mid-tail posterior diameter equals one-third anal body diameter. Cuticularized orifice of caudal glands (spinneret) 2 microns in length.

MALE: Not known.

NEOTYPE: Female collected September 1, 1952 by M. W. Allen and M. Oostenbrink, catalogue no. 123, University of California Nematode Survey Collection.

TYPE HABITAT: Soil around apple seedlings.

TYPE LOCALITY: Heiligerlee, the Netherlands.

Plectus longicaudatus is distinguished by the anteriorly directed cephalic setae, the length of the esophago-intestinal valve, and especially by the long slender tail, which usually assumes a ventral curve in the distal third.

Plectus meridionalis is placed in synonomy with *P. longicaudatus* because the only character difference is the "crown-like" margin described by Steiner. Examination of specimens shows this to be a common internal feature of the lip region of *Plectus*.

Plectus kenyanus is placed in synonomy because no characters are given which clearly distinguish it from *P. longicaudatus*. The distinguishing character given by Allgén is the tail length: C = 5.87-6.96. This measurement is within the range here given for *P. longicaudatus*: c = 4.7-7.1, and is close to the measurements originally given by Bütschli: c = approximately 6-7.

Specimens of this species were examined from: the United States, Alabama, California, Colorado, and New York; and from several localities in the Netherlands.

Plectus parvus Bastian, 1865 (Fig. 8, C-F)

Synonyms: *Plectus communis* Bütschli, 1873 (pro parte), pp. 91-92.
Plectus belgicae de Man, 1904b, pp. 10-12.
Plectus paracommunis Hoeppli, 1926, p. 238.
Plectus potamogeti Schneider, 1937, p. 65.
Plectus longicaudatus var. *opisthocirculus* Andrássy, 1952, p. 35.
Plectus opisthocirculus (Andrássy, 1952) Andrássy, 1958, p. 46.

Dimensions: 9 Females—L = .42-.51 mm., a = 17-22.4, b = 3.4-4, c = 7.9-10.4, V = $^{10\text{-}14}49\text{-}53^{11\text{-}15.5}$, Stoma = 11-17 microns. 1 Male—L = .48 mm., a = 22.4, b = 3.7, c = 8.2, T = 53%, Stoma = 12 microns.

Female: Body tapered little anteriorly, body almost cylindrical from excretory pore to anus, tapers rapidly from anus to tail tip. Six conoid-connate lips, height equals one-third width of lip region. Lip region not distinctly set off from body, lip region delineated by transverse striae of body. Four slender cephalic setae located two body annules behind the lip region. Cephalic seta length equals one-half width of lip region. Cuticle marked by fine transverse striae. Transverse striae interrupted on both sides of body by two longitudinal alae, each wing area occupies one-fifth of body width at vulva. Stoma almost cylindrical, length 11-17 microns, from anterior extremity slightly more than twice width of lip region. Cheilostom obscure, prostom one-third length of meso-metastom. Amphid diameter equals approximately one-sixth width of neck at their level. Amphids located at posterior one-half of stoma. Esophagus length variable from less than one-third to one-fourth of body length. Excretory pore at 65% of esophageal length. Nerve ring just anterior to excretory pore. Cervical papillae setiform, situated between longitudinal alae and just posterior to level of excretory pore. Length of esophago-intestinal valve approximately one-third of body width at its level. Vulva 49-53% of body length, usually equatorial or post-equatorial, vulval lips prominent. Eggs 21-25 microns × 46-48 microns. Tail length approximately five anal body diameters. Tail narrow, tapering; at anus, tail width one-half body width; one-fourth body width at mid-tail; and one-sixth of body width just anterior to tail tip. Cuticularized orifice of caudal glands (spinneret) 1.7-2 microns in length.

Male: Similar to female. Testes diorchic and opposed. Testes occupy 53% of body length. Spicules in same specimen asymmetrical in size and/or shape (13-19 microns). Spicules ventrally curved. Gubernaculum approximately one-third spicule length. Dorsal process of gubernaculum short, one-

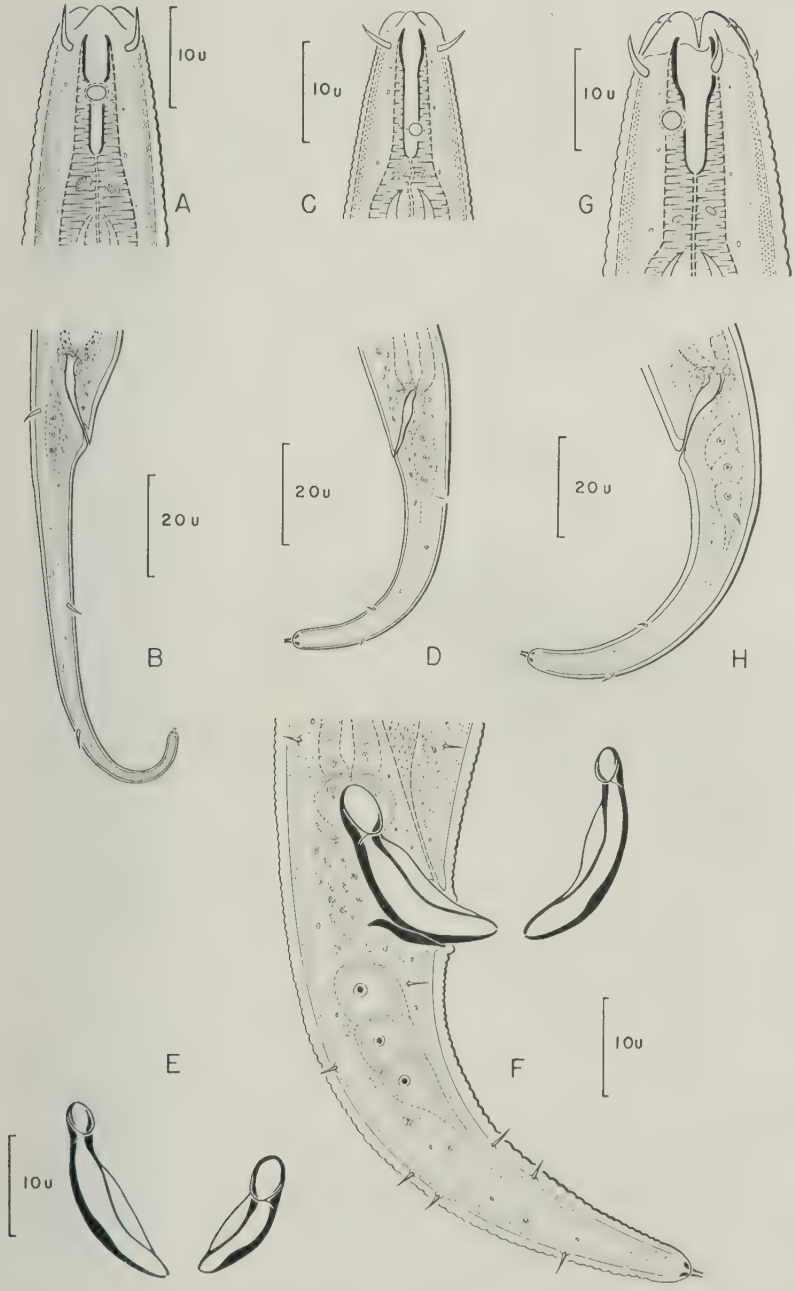

Fig. 8. A-B—*Plectus longicaudatus*. A—Head. B—Female tail. C-F—*Plectus parvus*. C—Head. D-Female tail. E—Spicules. F—Male tail. (E-F—Spicule variation.) G-H—*Plectus acuminatus*. G—Head. H—Female tail.

fourth of gubernaculum length. Caudal setae, seven on right side and eight on left. Preanal supplementary organs or seta missing.

TYPE HABITAT: Moss covering stone in fresh water stream.

TYPE LOCALITY: Balmouth, England.

Plectus parvus may be readily separated from other species of *Plectus* by the almost cylindrical stoma, posteriorly placed amphids, and its small size.

Two males were examined. However, the tail tip was missing from one male; therefore, measurements were given for only the one whole specimen. The other male was used for spicule size and shape.

Plectus communis (pro parte) is placed in synonomy because the original description embodies two species. The main description agrees with *P. parvus*, the remainder with *P. acuminatus*.

P. belgicae, *P. paracommunis*, *P. potamogeti* and *P. opisthocirculus* are placed in synonomy with *P. parvus* because on the basis of the original descriptions and illustrations they cannot be separated from *P. parvus*.

Specimens of this species were examined from: the United States, California, Colorado, and Utah; and from several localities in the Netherlands.

Plectus cornus, n. sp. (Fig. 6, F-G)

DIMENSIONS: 2 Females—L = .558-.59 mm., a = 26.5-29.5, b = 4.03-4.72, c = 7.02-7.35, V = $^{6.5\text{-}6.6}5$47.5-48.5$^{6\text{-}6.27}$, Stoma = 19 microns.

FEMALE (HOLOTYPE): L = .558 mm., a = 26.5, b = 4.03, c = 7.35, V = $^{6.5}$48.5$^{6.27}$, Stoma = 19 microns. Body slender, tapers to both extremities, most pronounced posteriorly. Cuticle marked by transverse striae. Striae pronounced on cephalic region and tail. Striations on both sides of body interrupted by two longitudinal alae. Lip region with six low, rounded, almost obscure lips. Lips not set off by a marked constriction. Four long, anteriorly directed cephalic setae, located on first body annule. Setae length 5 microns, approximately equal to width of lip region. Cheilostom obscure. Stoma length 19 microns, three times as long as width of lip region. Division between prostom and meso-metastom obscure, stoma almost cylindrical. Amphids located behind middle of stoma, 12.5 microns from anterior extremity Amphid diameter 2 microns. Esophagus approximately one-fourth of body length. Excretory pore ventral, located at 56.5% (56.5-73%) esophagus length. Nerve ring just anterior to excretory pore. Cervical papillae setiform, situated between longitudinal alae and at level of excretory pore. Esophago—intestinal valve long, length two-fifths of body width at its level. Vulva post-equatorial 48.5% (47.5-48.5%). Tail slender, almost cylindrical for most of its length, length approximately six anal body diameters. Cuticularized orifice of caudal glands (spinneret) 2 microns long.

MALE: Not known.

HOLOTYPE: Female collected August 11, 1959 by S. A. Sher, catalogue no. 203, University of California Nematode Survey Collection.

TYPE HABITAT: Soil from about grass.

TYPE LOCALITY: University of Uppsala, Uppsala, Sweden.

Plectus cornus is closely related to *Plectus parvus* but can be readily separated from it by the more cylindrical stoma, and obscurity of the prostom which exhibits little or no expansion and the longer heavier cephalic setae. *P. cornus* has six low rounded obscure lips as opposed to the conoid-connate lips of *P. parvus*.

Plectus cornus is also close to *P. armatus* and *P. assimilis* but is easily distinguished by the stoma shape and tail length.

Plectus acuminatus Bastian, 1865 (Fig. 8, G-H)

Synonym: *Plectus communis* Bütschli, 1873 (pro parte), pp. 91-92.

Dimensions: 17 Females—L = .577-.854 mm., a = 16.5-24.7, b = 2.77-4.38, c = 6.82-9.72, V = $^{8.5\text{-}13}46\text{-}52.1^{9\text{-}15}$, Stoma = 15.5-22.3 microns.

Female: Body short, plump, tapering to both extremities, most pronounced toward posterior. Cuticle marked by transverse striae, more pronounced on cephalic region and tail. Striae interrupted on both sides of body by two longitudinal alae, each wing area occupies one-tenth of body diameter at vulva. Lip region bears six conoid-connate lips, not distinctly set off from neck, lip region delineated by beginning of body annulations. Lips one-half as high as width of lip region. Four well developed (3-3.6 microns) cephalic setae, located two annules behind lip region. Stoma length 15.6-22.3 microns, equals one and one-half times width of lip region. Cheilostom indistinct, prostom two-thirds length of meso-metastom. Amphid 10.5-16 microns from anterior extremity, situated at region of meso-metastom. Amphid diameter 2-2.6 microns, approximately one-seventh neck diameter at their level. Esophagus approximately one-fourth of total body length. Excretory pore 50-61% of esophagus length. Nerve ring just anterior to excretory pore. Cervical papillae setiform, situated between longitudinal alae just posterior to excretory pore. Esophago-intestinal valve approximately one-fourth of body width at its level. Vulva equatorial, 46-52% of body from anterior extremity. Vagina extends into body one-third of body diameter at vulva. Eggs 27-31 microns × 48-62 microns. Rectum length 16-22 microns. Tail length little more than four anal body diameters, conoid and evenly tapered. Cuticularized orifice of caudal glands (spinneret) 2-3 microns.

Male: Not known.

Type habitat: Moss.

Type locality: England.

Plectus acuminatus is recognized by its size, relatively small amphid and the conoid shape of its tail.

Plectus communis Bütschli, 1873 (pro parte) is placed in synonomy with *P. acuminatus* because in Bütschli's original description two species are described. Bütschli recognized this fact. The larger of the two species described by Bütschli is here placed in synonomy with *P. acuminatus* of Bastian, 1865.

Specimens of this species were examined from the United States: California, Colorado and Wisconsin; other areas of the world: England, Israel, the Netherlands.

Plectus cirratus Bastian, 1865 (Fig. 9, A-B)

Synonym: *Plectus africanus* Dadai, 1908, pp. 15-16.

Dimensions: 13 Females—L = .92-1.5 mm., a = 18.8-26, b = 3.84-4.8, c = 7.6-9.4, V = $^{11\text{-}17}45\text{-}51^{11.5\text{-}17.5}$, Stoma = 20-26 microns.

Female: Body long, cylindrical, plump; tapering from anus pronounced, only slightly tapered anteriorly. Cuticle marked by fine transverse striations, striae on each side of body interrupted by two longitudinal alae. Each wing area occupies one-ninth of body diameter at vulva. Lip region rounded to truncate. Six conoid-rounded lips, height equals one-third width of lip region, lips not set off from neck. Four cephalic setae length 2-3 microns, setae located two annules behind lip region. Stoma length twice width of

lip region, stoma length 20-26 microns. Cheilostom distinct. Meso-metastom three times length of prostom. Amphid diameter 2-3 microns, diameter equals one-fifth width of neck at their level. Amphids 10-16.3 microns from anterior extremity, located at level of meso-metastom. Esophagus length approximately six times width of neck at posterior bulb. Excretory pore 48-55% of esophagus length. Nerve ring just anterior to excretory pore, cervical papillae setiform, situated between longitudinal alae and just posterior to level of excretory pore. Esophago-intestinal valve length one-third width of neck at its level. Vulva sometimes anteriorly located 45-51% of body length. Vagina extends into body one-third to one-half body diameter at vulva. Eggs 26-29 microns × 43-56 microns. Rectum length 23-32 microns. Tail length approximately five anal body diameters. Tail elongate conoid, cuticularized orifice of caudal glands (spinneret) 3-4 microns.

MALE: Not known.

TYPE HABITAT: About lower decaying leaves of *Myriophyllum verticillatum*, pond.

TYPE LOCALITY: Bagshot, England.

Plectus cirratus is similar to *Plectus palustris* de Man, 1880. It can be distinguished from the latter by the shorter tail, the shorter esophagus (in relation to neck width), the shorter esophago-intestinal valve, and the presence of four pairs of caudal setae.

Plectus africanus Dadai, 1908 is placed in synonomy with *Plectus cirratus* because the description given by Dadai agrees with that originally given by Bastian in 1865 and with the characters as they are here understood for *P. cirratus*.

Specimens of this species were examined from: the United States, California, Colorado, Idaho, and Utah; and from the Netherlands.

Plectus palustris de Man, 1880 (Fig. 9, C-E)

DIMENSIONS: 8 Females—L = 1.25-1.5 mm., a = 31.2-34, b = 43.-4.9, c = 7.2-7.9, V = $^{9\text{-}12}43\text{-}46^{9\text{-}13}$, Stoma = 23-26 microns.

FEMALE (NEOTYPE): L = 1.31 mm., a = 31.2, b = 4.6, c = 7.8, V = $^{10.5}44.4^{10.8}$, Stoma = 25.3 microns. Body long and slender, tapering only slightly to anterior, tapering pronounced from anus to tail tip. Cuticle marked by fine, indistinct transverse striations, striae on each side of body interrupted by two longitudinal alae, trough separating alae flat and equals one-third of longitudinal alae width. Each wing area occupies one-eighth of body diameter. Lip region almost truncate, lip region with six, low, rounded, connate lips. Lips one-third height of lip region width. Lips not set off from neck. Four long, 4 microns (3.66-4 microns) slender cephalic setae, located two to three annules behind lip region. Stoma twice as long as width of lip region, stoma length 25.3 microns (23-26 microns). Cheilostom visible but indistinct. Prostom one-third length of meso-metastom, prostom only slightly expanded. Amphid diameter 3 microns (3-3.3 microns) equals one-fourth of width of neck at this level. Amphids 15.5 microns (13.5-17.5 microns) from anterior extremity located at level of meso-metastom. Esophagus length more than seven times width of neck at posterior bulb. Excretory pore 52% (48-55.5%) of esophagus length. Nerve ring one body width anterior to excretory pore. Cervical papillae setiform, situated between longitudinal ridges posterior to level of excretory pore. Esophago-intestinal valve equals one-half body diameter at its level. Vulva anteriorly

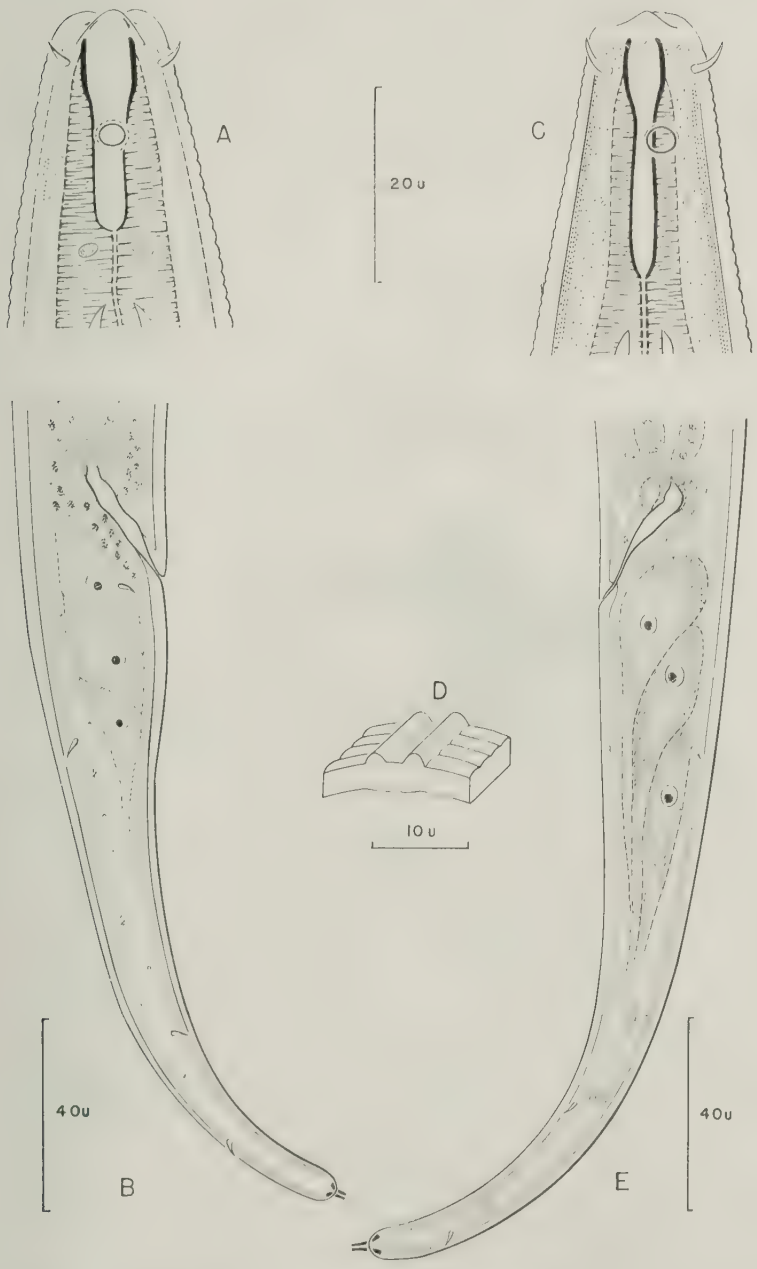

Fig. 9. A-B—*Plectus cirratus*. A—Head. B Female tail. C-E -*Plectus palustris*. C—Head. D—Longitudinal alae. E—Female tail.

located on body 44.4% (43.5-46%) total body length. Vagina extends into body one-half body diameter at vulva. Rectum length 29.5 microns (26-31.6 microns). Anal body diameter three-fourths diameter of body at vulva. Tail length slightly more than six anal body diameters. Tail tapers evenly to tail tip, tail diameter at tip one-third anal body diameter. Cuticularized orifice of caudal glands (spinneret) 4 microns (3.3-4 microns) in length.

Male: Not known.

Neotype: Female collected September 22, 1952 by M. W. Allen and M. Oostenbrink, catalogue no. 121, University of California Nematode Survey Collection.

Type habitat: Soil in meadow along the Rhine River.

Type locality: Wageningen, the Netherlands.

Plectus palustris is distinguished from *Plectus cirratus* Bastian, 1865 by the three pairs of caudal setae, the longer, more slender tail, and by the two separated longitudinal alae (Plate 9, D).

Specimens of this species were examined from the following locality and habitat: meadow on the banks of the Rhine, Wageningen, the Netherlands.

Other species reported in the Genus *Plectus* Bastian, 1865

Species inquirendae: *Plectus tenuis* Bastian, 1865
Plectus tritici Bastian, 1865
Plectus rivalis (Dujardin) Bastian, 1865
Plectus demani Orley, 1880
Plectus geophilus de Man, 1880
Plectus insignis Cobb, 1893a
Plectus minimus Cobb, 1893b
Plectus pusillus Cobb, 1893b
Plectus agilior Cobb, 1898
Plectus pygmaeus
syn: *Pycnolaimus pygmaeus* Cobb, 1920
Plectus aberrans Kreis, 1930 (poss. *Paraplectonema*)
Plectus amphidiscatus Filipjev, 1930
Plectus paraguayensis Kreis, 1932
Plectus chengmoghliangi Hoeppli and Chu, 1932
Plectus effilatus Schuurmans Stekhoven and Mawson, 1954
Nomen dubium: *Plectus triplogaster* Örley, 1880
Plectus elymi Allgén, 1949
Plectus grahami Allgén, 1951
Plectus gisleni Allgén, 1951
Plectus frigophilus Kiryanova, 1958
Plectus globilabiatus Kiryanova, 1958
Species formerly listed in *Plectus* Bastian, 1865:
Anaplectus de Coninck and Schuurmans Stekhoven, 1933.
A. granulosus (Bastian, 1865) de Coninck and Schuurmans Stekhoven, 1933.
Synonymy:
P. blanci Höfmanner and Menzel, 1914
P. schneideri de Man, 1880
P. tubifer Cobb, 1914
A. submersus (Hirschmann, 1952), n. comb.
Paraplectonema Strand, 1934
P. pedunculatus (Höfmanner, 1913) Strand, 1934

Wilsonema Cobb, 1913
W. auriculatum (Bütschli, 1873) Cobb, 1913
W. cephalatum (Cobb, 1893) Cobb, 1913
W. fausti (Kreis, 1930) Goodey, 1951
W. otophorum (de Man, 1880) Cobb, 1913
W. tentaculatus (Fuchs, 1930) Schneider, 1939
Acrobeloides (Cobb, 1924) Thorne, 1937
A. minor (Thorne, 1925) Thorne, 1937
Synonym:
P. obtusicaudatus Dadai, 1901
Chronogaster Cobb, 1913
C. multitubiferus (Imamura, 1931), n. comb.
Synonym:
P. multitubiferus Imamura, 1931

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Lauratonema obtusicaudatum, n. sp. (Nemata: Enoploidea), A Marine Nematode from the Coast of Oregon*

D. G. MURPHY and H. J. JENSEN

In 1953 Gerlach described the genus *Lauratonema,* for which he erected the family Lauratonematide. One of the outstaning characteristics of the family was the presence of a cloaca formed by the female genital tract opening into the rectum. At that time Gerlach described *L. reductum* and *L. adriaticum* from Palermo, Sicily, and Rimini, Italy respectively. *L. hospitum* Gerlach was described from Santos, Brazil in 1954. He described L. *originale* from Kiel Bay, Germany in 1956. This nematode closely resembles the type species, *L. reductum,* of the genus in many respects, but differs significantly in that rather than having a cloaca, the vulva opens anterior to the anus. *L. spiculifer* Gerlach 1959 was described from Abd-el-Kuri Island in the Gulf of Aden. In 1959 Wieser described *L. mentalatum* and *L. pugiunculus* from Puget Sound, Washington, on the basis of male specimens only.

Since one genus usually does not admit divergent female characters accommodating species with either a cloaca or separate anal and vulvular openings, a new genus should eventually be established for members of the family that possess a vulva. The two species described by Wieser cannot be definitely placed until females are described or distinguishing male characters are established. Such characters may be present in the buccal cavity structure, which is broader with heavier sclerotization of the stomal walls in the cases of *L. originale* Gerlach 1956 and *L. mentulatum* Wieser 1959 than in other representatives of the family.

The species described here fits the original description of the genus *Lauratonema* Gerlach 1953.

Lauratonema obtusicaudatum, n. sp.

Female:	L = 1.20mm.	a = 50.1	b = 5.0	c = 6.7	ovary = 36.3%
Male:	L = 1.30mm.	a = 53.1	b = 5.0	c = 11.6	
Male:	L = 1.24mm.	a = 56.4	b = 5.1	c = 10.7	
Male:	L = 1.27mm.	a = 54.2	b = 5.1	c = 11.2	
Male:	L = 1.27mm.	a = 55.9	b = 5.1	c = 11.8	

Body with fine, distinct annulation. Few very fine somatic setae. Cephalic region without annulation, set off by a slight constriction. Six conical labial papillae. Lips obsecure. Ten cephalic setae 11 microns and 5.6 microns long. Amphids indistinct, perhaps shepherd's crook. Stoma funnel shaped, having two weakly sclerotized transverse ridges. Esophagus cylindrical with slight swellings anteriorly and posteriorly. Excretory pore not observed. Monodelphic. Both sexes with cloaca. Males with one preanal and two postanal papilloid structures in which neither nerves nor pores could be observed. Spicula 19 microns long, acute bend distally, weakly sclerotized. Gubernaculum not observed. Tail cylindrical, possessing several setae. Tails of relaxed male specimens characteristically bent sharply at 90° angle.

HOLOTYPE: male collected 21 August 1958 by D. G. Murphy, slide OSC OM 5A, Oregon State College collection.

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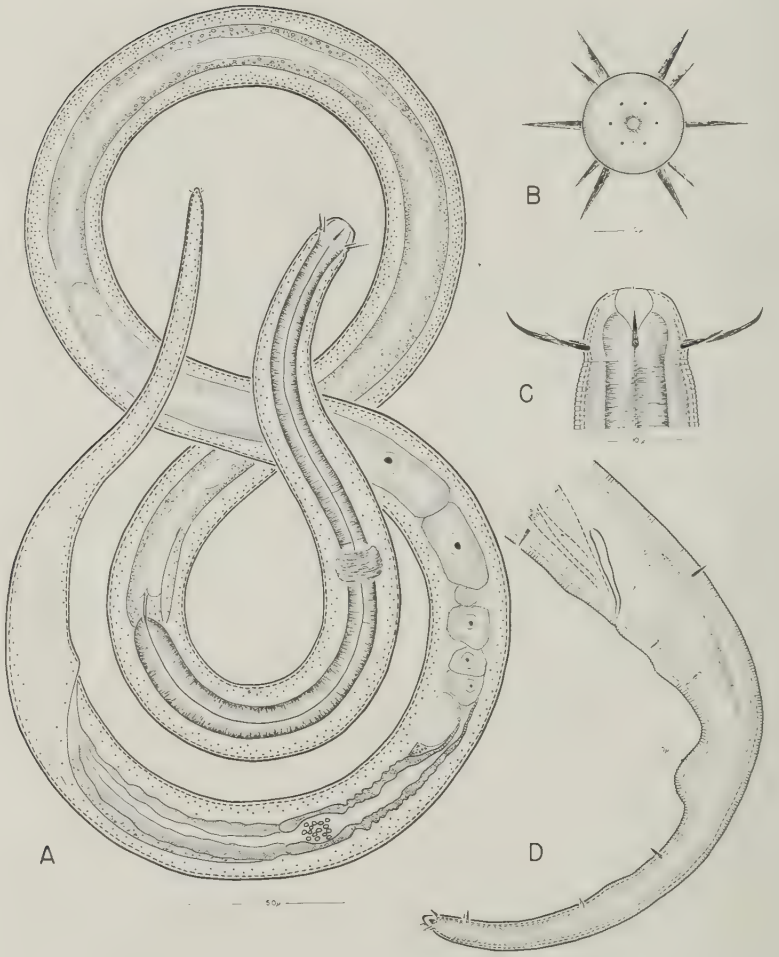

Fig. 1. *Lauratonema obtusicaudatum* n. sp. A. Female; B. Male face view; C. Male lateral head view; D. Male tail.

ALLOTYPE: 1♀, OSC OM 5B.

PARATYPES: 3♂, OSC OM 5A.

TYPE-LOCALITY: South Slough, Charleston, Oregon, subtidal.

DIAGNOSIS: Resembling *L. reductum* Gerlach 1953 but smaller, differing primarily in the presence of papilloid structures on male caudal region, and possession of a slight cervical constriction.

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The "Hemizonion" an Unrecorded Companion Structure of the Cephalids and the Hemizonid*

FIELDS E. CAVENESS**

The hemizonid has been described in many members of the phylum Nematoda since the first report of this structure by Goodey (1951). Timm (1960) suggests the universal presence of the hemizonid throughout the phylum. Hirschmann (1956, 1959) reported and described similar anterior structures termed "cephalids" from the genera *Heterodera* and *Hoplolaimus*.

The hemizonid is situated on the ventral aspect of the nematode body, usually in the region of the excretory pore. In longitudinal view the hemizonid appears more or less lenticular, more highly refractive than adjacent parts and terminates at the lateral chords.

The cephalids, anterior and posterior, are highly refractive structures, lenticular in longitudinal section and circumscribe the nematode cephalic region.

In this paper a structure similar to the hemizonid, with some of the characteristics of the cephalids and apparently heretofore undescribed is reported.

The structure, apparently a diminutive mimic of the hemizonid, was found on members of the super-families Rhabditoidea, Tylenchoidea, Aphelenchoidea and Dorylaimoidea. This structure is located on the ventral side of the nematode body generally at the level of the basal bulb in the Secernentea (Phasmidia) examined. Its position varying in different forms from the anterior margin to the base of the basal bulb. It appeared immediately posterior to the flanged stylet extension in *Xiphinema ebriense* Luc, 1958, of the class Adenophorea (Aphasmidia). Apparently it is always situated posteriad to the hemizonid. It appears as a small, bright, lenticular structure and is more highly refractive than adjacent parts.

By examination of various specimens from ventral to lateral positions this new structure was found to be of a hemizonidal nature. It is similar in structure, being bandlike, more or less biconvex to flat in section and located between the cuticle and the hypodermal layer. It extends around the ventral portion of the nematode terminating, as does the hemizonid, just short of the lateral field on either side.

The term "Hemizonion" is proposed as the name for this structure. The term being the diminutive of hemizonid; hence a "small hemizonid."

Goodey (1959) reported observations which reveal the hemizonid to be functional as a nerve commissure. Probably a detailed study of the hemizonion will disclose a similar function. A minute examination of the nematode anatomy may reveal a series or a system of such structures.

The hemizonion was best observed on newly relaxed specimens although it is readily detected on forms mounted in glycerine. In *Hoplolaimus propori-*

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cus Goodey, 1957, the hemizonion appears 5 to 12 annules posterior to the hemizonid (Fig. 1A). It is about one-half annule wide compared to about two and one-half annules wide for the hemizonid and is proportionately more shallow. The hemizonion is 7 annules posterior to the hemizonid in *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941 (Fig. 1B). It is about one annule wide compared to about two and one-half annules wide for the hemizonid. In *X. ebriense* it is about 30 microns posterior to the hemizonid and is about one-third the size.

The hemizonion has been observed by the author in the following: *Neocephalobus aberrans* (Steiner, 1929) Steiner, 1934; *Acrobeloides* sp.; *Acrobeles complexus* Thorne, 1925; *Tylenchus filiformis* Butschli, 1873; *Tylenchorhynchus martini* Fielding, 1956; *Hoplolaimus proporicus* Goodey, 1957; *Rotylenchus robustus* (de Man, 1880) Filipjev, 1945; *Pratylenchus brachyurus* and (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941; *Trophurus minnesotensis* (Caveness, 1958) Caveness, 1959; *Aphelenchus avenae* Bastian, 1865; *Paraphelenchus pseudoparietinus* (Micoletzky, 1922) Micoletzky, 1925; *Dorylaimus* spp.; and *Xiphinema abriense* Luc, 1958.

Hirschmann (1960) reports the presence of a hemizonion in the genera *Scutellonema, Anguina* and *Ditylenchus.*

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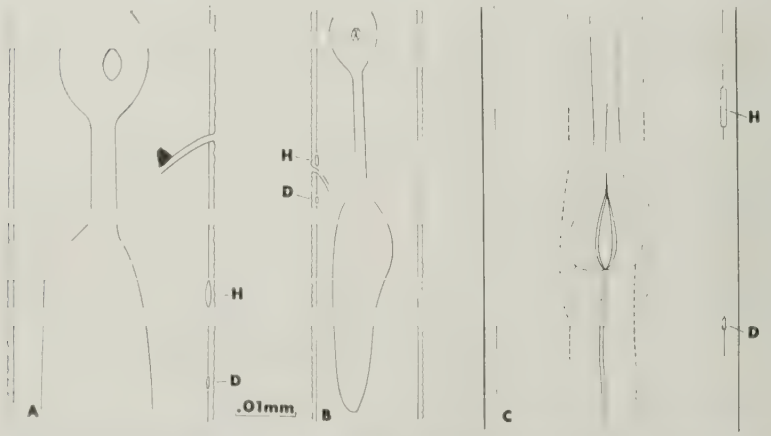

Figure 1. Portions of esophagi showing the relative positions of the hemizonid and hemizonion. A. *Hoplolaimus proporicus*, B. *Pratylenchus brachyurus*, C. *Xiphinema ebriense*. D. Hemizonion. H. Hemizonid.

The Accumulation of Plant Parasitic Nematode Larvae around Carbon Dioxide and Oxygen

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The general attraction and relationship of phytoparasitic nematode larvae to plant roots and chemical agents emitted by them has been studied by Bird (1959), Linford (1939), Lownsbery & Viglierchio (1958, 1960, 1961), Peacock (1959), Wieser (1955), Wallace (1958), Viglierchio & Lownsbery (1957, 1960), and Viglierchio (1961). Kühn (1959) using *Heterodera rostochiensis* larvae suggests that accumulation of larvae around plant roots is due to random movement and detention of the larvae near the roots by chemical agents. Lownsbery & Viglierchio (1961) experimenting with *Meloidogyne hapla* larvae give evidence that accumulation around seedlings involves, in addition to random movement, a response to a dialyzable agent. In addition to work with plant roots Klinger (1959), Bird (1959, 1960) and Rohde (1960) have investigated the influence of carbon dioxide as an attractant.

This is a study of the effect of two naturally occurring gases in the accumulation of plant parasitic nematode larvae.

Methods and Materials

Infective larvae of *Meloidogyne javanica*, *M. hapla* and *Heterodera schachtii* used in these experiments were obtained using the method described by Lownsbery & Viglierchio (1961). *Ditylenchus dipsaci* larvae from garlic scales were obtained in a similar fashion.

Experiments were conducted in a channel of extruded aluminum which was coated with asphaltum paint to avoid aluminum toxicity. Both sides, the entire length of the channel, were inscribed with marks spaced at 1 cm intervals. Aluminum plates placed at the ends of the channel which had been precoated with lanolin prevented loss of water. A spring and chain arrangement around the entire channel secured the plates in position.

Nematode larvae were concentrated by centrifugation and suspended in 500 cc of boiled distilled water. The volume of larvae and water was evenly distributed in the channel and sand was added until only a thin film of water remained.

Dialysis tubing 1 cm in width (previously leached for a 72-hour period) was filled with boiled distilled water, boiled distilled water enriched with carbon dioxide, and boiled distilled water enriched with oxygen. Each tube was placed in the center of an 11 cm testing unit so that it occupied a 1 cm section of the sand with five 1 cm sections on either side. In each trial four replications were prepared for each of the two gases and the water checks tested. A thin layer of sand was then sprinkled over the unit to cover the tubes and take up excess moisture. However, enough water remained in the unit to completely fill the pore spaces and give a slurry consistency to the sand.

After approximately 24 hours the testing units were prepared for sectioning by adding dry sand until the media became firm (yet remained well wet) for slicing. The testing unit was sliced into 1 cm sections and each section was placed on a modified Baermann funnel (Goodey, 1957) to recover the

larvae. In this manner it was possible to establish the ratio between the total number recovered and those recovered from each 1 cm section. At least 70 percent of the total number of larvae added (90,000-150,000 per 11 cm testing unit) were recovered by this method. Slicing of the testing unit into 1 cm sections was accomplished in the same manner as described by Viglierchio (1961).

Water was prepared for enrichment by first boiling for approximately one hour to remove soluble gases. It was then divided into three portions; one to be enriched with carbon dioxide, another with oxygen and the third portion to be used as a check. The boiled distilled water to be enriched with carbon dioxide was placed in a receiving tube having an inlet and outlet valve. Dry ice was used as a supply of carbon dioxide gas which was scrubbed with concentrated sulfuric acid and moistened by being run through boiled distilled water (at room temperature) and subsequently introduced into the water in the receiving tube by a gas disperser attached to the inlet tube. This process was continued for at least four hours while the temperature of the water was maintained at 1°C.

The apparatus for enriching water with oxygen was the same as that used for carbon dioxide except that the scrubbing process with concentrated sulfuric acid was eliminated and oxygen obtained from a cylinder was bubbled into boiled distilled water, then introduced into the receiving tube maintained at 1°C.

Results and Discussion

Boiled distilled water enriched with carbon dioxide gas is a collecting zone for the accumulation of *M. hapla* larvae around the tube section in the test-

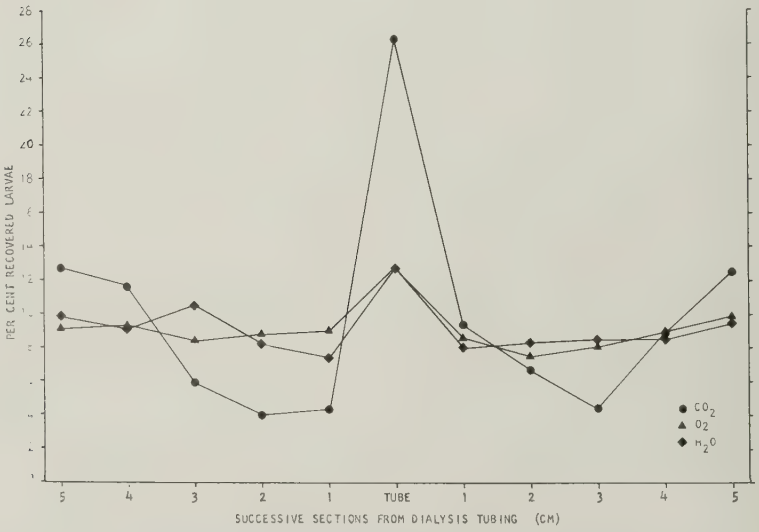

Figure 1. The distribution of *Meloidogyne hapla* larvae after a 24-hour exposure to water enriched with carbon dioxide and oxygen gas and boiled distilled water checks. (The CO_2 peak in the figure is significantly different from O_2 and H_2O checks at the 1 percent level.)

ing unit (Fig. 1). Larvae accumulated in the tube sections by migrating from adjacent sections as indicated by the valleys adjacent to the peak. The sections of sand containing tubes of oxygen enriched water and water checks did not exhibit significant accumulation since the theoretical percent recovered larvae for each of the sections was approximately 9.2. From the above information it appears unlikely that the dialysis tubing has any effect on accumulation. Experiments were also conducted using *M. javanica* larvae which demonstrate the same type curve as that shown for *M. hapla*.

Bird (1959) using *M. hapla* larvae was unable to demonstrate any significant attraction to discs of agar through which carbon dioxide gas had been bubbled previously while in its molten state. Apparently this observation results from the reduced solubility of carbon dioxide at that temperature.

Infective larvae of *H. schachtii* (Fig. 2) accumulate around the carbon dioxide section and also show an equal accumulation around oxygen. The curve for the water checks demonstrates no accumulation within the unit but maintains a level near the theoretical distribution of larvae per 1 cm section (9.2 percent). *D. dipsaci* larvae were also used as experimental animals, and they reacted in a similar manner to *H. schachtii*.

Results of these tests with *D. dipsaci* support observations made by Klinger (1959). He observed the accumulation of *D. dipsaci* larvae at the source of carbon dioxide by introducing the gas into a watch glass through a capillary glass tube and watching the larvae as they accumulated around and within the tube. More recently Bird (1960) using the Klinger technique which sustains a higher carbon dioxide concentration gradient than Bird's original method, reported the accumulation of larvae of *M. javanica*, *H. schachtii*, larvae and adults of *Pratylenchus minyus*, pre-adult larvae of *Paratylenchus* sp. and various rhabditids about the carbon dioxide capillary source. Results of present experiments conducted with *D. dipsaci* and *H. schachtii* show an equal accumulation of the larvae near oxygen.

The possibility was considered that acidity could be a factor in accumulation. The hydrogen ion concentration of water remaining in the dialysis

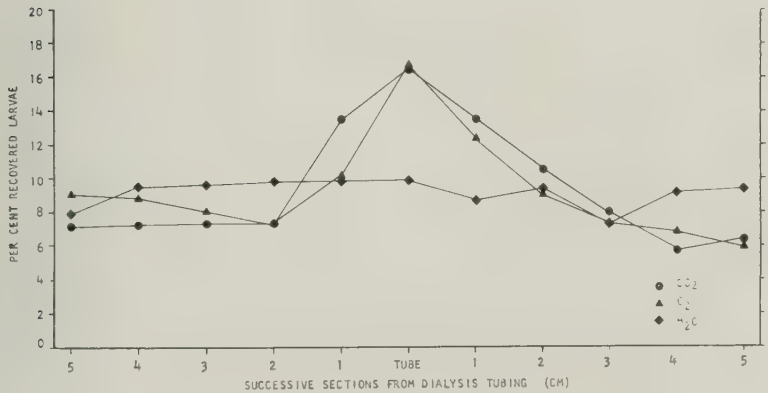

Figure 2. The distribution of *Heterodera schachtii* larvae after a 24-hour exposure to water enriched with carbon dioxide and oxygen gas and boiled distilled water checks. (The CO_2 and O_2 peaks in the figure are significantly different from H_2O checks at the 1 percent level.)

tubing and of sand from sections 1-5 was determined after 24 hours and larvae corresponding to these sections were counted. No correlation between acidity and accumulation was noted.

Kühn (1959) and Rohde (1960) suggested that accumulation around a source of carbon dioxide was a result of random movement and detention of the larvae in the area occupied by the gas. Kämpfe (1959) using *H. schachtii* larvae was able to demonstrate (1) the arrest of movement as a direct result of carbon dioxide and (2) greatly increased activity of the larvae following the treatment period. In view of the data presented here using carbon dioxide and oxygen and observations by Klinger and Bird with carbon dioxide, it is apparent that additional explorations for mechanisms of accumulation will be necessary.

Summary

The accumulation of some plant parasitic species of nematode larvae to carbon dioxide and oxygen has been demonstrated experimentally. *Ditylenchus dipsaci, Meloidogyne hapla, M. javanica,* and *Heterodera schachtii* all accumulated around a source of carbon dioxide. *H. schachtii* and *D. dipsaci* larvae accumulated around a source of oxygen as well as carbon dioxide.

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The American Otter, *Lutra canadensis vaga*, as a Host for Two Species of Trematodes Previously Unreported from North America*

THOMAS K. SAWYER

During a study of internal parasites of south Georgia wildlife in 1955, the viscera and internal organs were examined from one American Otter, *Lutra canadensis vaga*. Two species of trematodes which are believed to be unreported from North America were found in the digestive tract: *Baschkirovitrema incrassatum* (Diesing, 1850) Skrjabin, 1944, from the stomach, and *Enhydridiplostomum alarioides* (Dubois, 1937) Dubois, 1944, from the small intestine. *B. incrassatum* has been reported only from Brazil (Diesing, 1850; Braun, 1901), and from Southern Rhodesia (Beverley-Burton, 1960). *E. alarioides* has not been reported since its original description from the Brazilian otter, *Lutra brasiliensis* (Dubois, 1937). Although *B. incrassatum* has not been reported from North America the Helminth Collection at the U.S.D.A. Laboratory of Parasitology, Beltsville, Maryland did contain several specimens collected by Dr. W. J. Hamilton in New York (Nos. 45943, 49081, 49082).

The occurrence of *B. incrassatum* in North America is of interest particularly from a zoogeographical point of view. This parasite is reported only from various species of otter and there are only three reports since the original description over one hundred years ago. The paucity of reports of this species may be attributed to its apparent host specificity and secondly, to its occurrence in a host infrequently available for parasitological examination.

The morphological characteristics of *E. alarioides* are in general agreement with the subfamily Diplostomatinae (Parasites of Birds), but not with the subfamily Alariinae (Parasites of Mammals), as provided by Dubois (1937, 1938, 1953). The rigid separation of these two subfamilies according to avian or mammalian hosts has imposed serious taxonomic limitations on the classification of new genera during the past twenty years, particularly among parasites of the Mustelidae. McIntosh (1939, 1940), originally described *E. fosteri* from the Panama otter, *Lutra repanda*, as a member of the genus *Diplostomum* (subfamily Diplostominae), and at the same time pointed out the marked resemblance of this species to other parasites of the otter in the genus *Alaria* (subfamily Alariinae). Subsequently Chandler and Rausch (1946) reported new species of *Alaria* and *Fibricola* from Michigan mammals which were incompatible with the systematics of Dubois. These authors proposed that the subfamily Alariinae be suppressed and that members of the genus *Alaria* reported from otters be included in the genus *Enhydridiplostomum*. More recently, Beverley-Burton (1960) described the new genus *Prudhoella* from Rhodesian otters. This author was unable to assign this genus to the subfamily Alarinae as required by Dubois and proposed that the revisions of Chandler and Rausch (1946) be accepted. In the present

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work the same taxonomic problems reported by Chandler and Rausch (1946), and Beverley-Burton (1960) were encountered with *Enhydridiplostomum* from the American otter. It is suggested that the subfamily Alariinae be suppressed as proposed by Chandler and Rausch (1946) or that new criteria be established to retain this subfamily. Distinctions among the Diplostomatidae on the basis of an avian or mammalian host could possibly be included at the generic or species level.

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Effect of Population Density on Adult Worm Survival in Primary *Nippostrongylus brasiliensis* Infections in the Rat

A. James Haley and John C. Parker*

Several investigators have reported the occurrence of a crisis early in the course of a primary infection of *Nippostrongylus brasiliensis* (Travassos, 1914) in the laboratory rat (Africa, 1931; Chandler, 1932; Graham, 1934; Porter, 1935; Sarles, 1939; Taliaferro and Sarles, 1939; Donaldson and Otto, 1946; Watt, 1943; Haley, 1958; Hurley, 1959). The decreased egg production and loss of worms at this time was attributed, by these authors, to the development of an acquired immunity by the host. A comparison of data from some of these studies suggested that the rate of loss of adult *N. brasiliensis* may have been influenced by the number of worms initially present in the gut. The purpose of the present work was to test this point by making a direct comparison of the rates of loss of adult worms from populations of different initial densities.

Materials and Methods

Different groups of Sprague-Dawley rats, 7 to 9 weeks of age, were inoculated intracutaneously with low, high and intermediate doses of *N. brasiliensis* larvae, and portions of each group were killed 10, 20 and 30 days later and examined for adult worms.

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Equal numbers of male and female rats were used in each experiment. The strain of *N. brasiliensis* used has been maintained in Sprague-Dawley rats for many years, and the procedures for handling it were the same as those described by Haley (1958).

Results and Discussion

Three experiments were performed in order to determine the average longevity of *N. brasiliensis* adults under different conditions of initial adult worm population density. In each case it was clear that adult worms survived longest in animals that had the smallest initial worm burdens (Table 1, Fig. 1).

This study has shown that the rate of loss of *N. brasiliensis* from the gut in primary infections of laboratory rats is intimately related to the size of the initial adult worm population. The loss of worms from rats with small initial worm populations was gradual over the 30 day period, whereas in hosts with high or intermediate initial worm burdens there was a marked reduction in population size during the same period of time. Rats that had a mean of about 23 worms on day 10 still retained about 80 per cent of this population on day 20 and 63 per cent on day 30. This is in striking contrast to rats that harbored mean burdens of about 1040 on day 10 but retained less than 3 per cent of this population by day 20. Animals whose initial worm burdens were between these extremes showed intermediate pictures.

Prior to this work there was only limited and indirect information on the influence of worm population size upon the dynamics of adult *N. brasiliensis* populations. Africa (1931) and Haley (1958) both noted that rats given about 500 larvae lost over 75 per cent of their adult worms during the late second and early third weeks of infection. Chandler (1932) claimed that there was no appreciable loss of worms from animals that received 200 larvae until the end of about three weeks, after which there was a drop to about 50 per cent of the original infection during the next three weeks. According to Hurley (1959), rats inoculated with just 20 larvae still had 30 to 40 per cent worm recoveries in the fourth week of infection. A similar result was obtained in the present study when a dose of 50 larvae was given.

Africa (1931) stated that egg production fell off more rapidly in rats infected with 500 larvae than in those given 200 larvae. He suggested that heavy infections of *N. brasiliensis* were eliminated by rats more quickly than moderate infections. Graham (1934) also found that the rate at which egg production ultimately decreased was related more or less directly to the number of *N. brasiliensis* larvae given to the rats.

There is very little information on the effect of adult worm population density on the longevity of other species of nematodes. Sandground (1936) reviewed the subject of longevity of various helminths but did not consider factors that may influence it. Dorman (1928) reported that the percentage recovery of *Heterakis papillosus* was greater in chickens given low doses than in those that received high doses of the parasite. In *Ascaridia galli* infections of chickens the percentage recovery of adult worms on the 21st day of infection was inversely related to the number of eggs administered (Ackert *et al*, 1931). Sadun (1949) reported that chickens given high doses of *Ascaridia galli* harbored a smaller percentage of worms on the 47th day of infection than those given low doses. In *Trichinella spiralis* infections of hamsters, the greatest loss of adult worms occurred between the 3rd and 5th days, but the percentage loss from high initial populations did not appear

to be different from that of low initial populations (Sadun and Norman, 1956).

The development of an acquired immunity by rats repeatedly infected with *N. brasiliensis* is well known. Evidence for an antibody basis of this immunity has been presented by several investigators (Sarles and Taliaferro, 1936; Chandler, 1938; Sarles, 1938, 1939; Matsumori and Miyasaka, 1942; Stewart, 1951; Thorson, 1951, 1953a, 1953b, 1954a, 1954b; Jackson, 1960). All of these studies were performed using sera from animals that had received large multiple doses of worms or worm products. A number of workers using various criteria, i.e. ability of larvae to complete the somatic migration, growth of worms, egg and worm counts, have presented evidence for an enhanced resistance in rats following a single exposure to *N. brasiliensis* (Africa, 1931; Schwartz, Alicata and Lucker, 1931; Chandler, 1932; Graham, 1934; Spindler, 1934, 1936; Porter, 1935; Hurley, 1959). To the writers' knowledge, however, no one has demonstrated antibody in a primary *N. brasiliensis* infection. The only known published work on this point is a note by Stewart (1951) in which sera from rats with primary infections were negative in complement-fixation tests with antigens prepared from adult *N. brasiliensis*.

Due to lack of serological data on primary infections it is not possible at this time to interpret directly the present findings with respect to the host's immune response. It seems likely, however, that the relationship between initial worm population density and the rate and extent of worm loss reflects different degrees of immune response by the host in each case.

SUMMARY

The longevity of adult *N. brasiliensis* in primary infections of laboratory

Table 1. Comparison of mean recoveries of adult *Nippostrongylus brasiliensis* from rats 10, 20 and 30 days after intracutaneous inoculation with different numbers of filariform larvae.

Experiment No.	Host Group	No. of Larvae Given	No. of Rats	Duration of Infection in Days	Percentage Recovery as Adult Worms	
					Range	Mean
I	A	50	4	10	20 –56	44
			4	20	16 –46	33
			4	30	0 –50	24
	B	2155	4	10	43 –50	48
			4	20	0 – 5	2
			4	30	0.2– 8	3
II	A	75	4	10	48 –76	61
			4	20	17 –59	32
			4	30	3 –25	11
	B	320	4	10	46 –58	53
			4	20	0.3–14	5
			4	30	0.9– 4	2
	C	1620	4	10	60 –67	65
			4	20	0.6– 1	0.8
			4	30	0.3– 2	1
III	A	50	6	10	26 –72	49
			6	20	36 –56	42
			6	30	20 –50	35
	B	275	6	10	54 –77	68
			6	20	2 –33	18
			6	30	3 –32	15

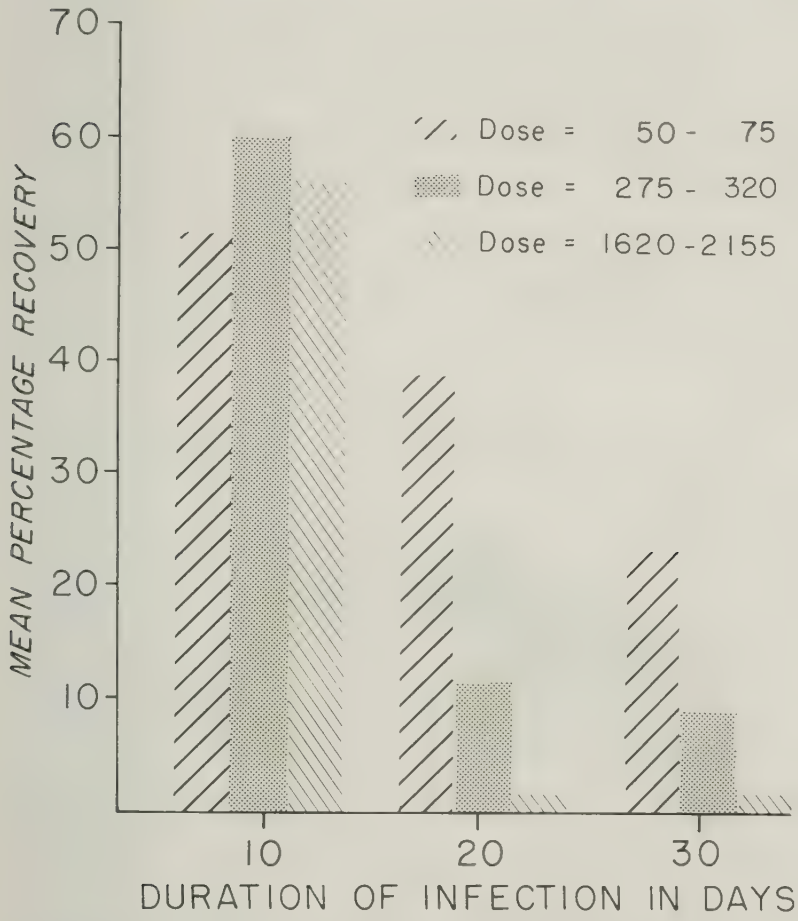

Fig. 1. Mean percentage recoveries as adult *N. brasiliensis* from different groups of rats 10, 20 and 30 days after intracutaneous inoculation with low, intermediate and high doses of filariform larvae.

rats was shown to be related to the size of the initial worm population. The loss of worms from small initial populations was gradual over a period of 30 days, whereas when high or intermediate initial worm burdens were present there was an abrupt loss in much less time. It is suggested that the relationship between initial worm population density and the rate and extent of worm loss reflects different degrees of immune response by the host in each case.

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Survival of the Infective Larvae of *Cooperia punctata* of Cattle on Pasture in Hawaii*

JOSEPH E. ALICATA

Goldberg and Rubin (1956) and Goldberg and Lucker (1959) have published results of experiments on the survival of larvae of gastrointestinal nematodes of cattle, including *Cooperia punctata*. The above studies were carried out under climatic conditions prevailing near Beltsville, Maryland which included the heat and dryness of the summer months and the cold of the winter months. The present study was carried out in Hawaii where mild climatic conditions are known to prevail. Since these parasites are common among calves in this area (Alicata, 1960), it is believed that the results of this study would be useful in formulating pasture management practices for their control.

MATERIALS AND METHODS

The present tests were conducted in two separate low-lying pastures on the Island of Oahu. Pasture A (Kawailoa Dairy), has an average annual rainfall of 36.8 inches, and pasture B (Valley Dairy), an average annual rainfall of 82.8 inches. Most of the grass in pasture A consisted of Bermuda grass (*Cynodon dactylon*) and in pasture B, pangola grass (*Digitaria decumbens*). The procedure in each pasture was to fence off a small, level and unshaded grassy area about 6 by 6 feet, and leave it unused for about 5 months. Within each area 6 galvanized iron ring-bands, 7.5 inches in diameter and 3 inches high, were then driven into the ground to a depth of about 2 inches Each ring-band surrounded a clump of grass. The grass of each of these isolated small plots was first clipped to a height of about 5 inches and then about ½ million infective *C. punctata* larvae, freshly isolated from 8- to 10-day-old fecal cultures, were distributed at the base of the grass. The grass outside the ring-bands was kept clipped to a height of about 3 inches. At monthly intervals the contaminated grass and approximately 1 inch of top soil were removed and examined for larvae with the use of the Baermann apparatus, leaving the samples in the Baermann funnels for a period of 24 hours. The number of larvae recovered from each contaminated area was estimated by dilution counts and then fed to a young rabbit. About 15 days later the rabbit was sacrificed and the small intestine examined for adult worms. As the contaminated grass from each of the above small plots grew taller, the upper portion was tied and supported by a narrow wooden pole driven into the ground.

In the above study, the first experiment was carried out during the months of June to November when the amount of rainfall in this area is usually low. A second similar experiment was carried out within the same fenced area during January to June when the amount of rainfall is usually higher. Because of the mild and more or less uniform local climatic conditions the year-round, no temperature records were maintained. However, it can be stated that at low altitude in Honolulu, the temperature is known to range from 66.6 to 82.9°F. with a yearly average of 74.9° F.

RESULTS AND DISCUSSION

Table 1 summarizes the number of *Cooperia* larvae which were recovered

*From the Department of Parasitology, Hawaii Agricultural Experiment Station, University of Hawaii, Honolulu.

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monthly from each of the small grass plots which had originally been contaminated with approximately ½ million larvae. These data show that the number of larvae which were recovered was the highest in the first month after the date of contamination. In the subsequent months the number of larvae recovered was drastically reduced. In pasture A when the monthly rainfall averaged 1.46 inches some of the larvae survived up to 3 months, but in another trial when the rainfall averaged 2.42 inches a few of the larvae survived up to 4 months. In pasture B when the average monthly rainfall was 3.85 inches some of the larvae survived up to 4 months, but in another trial when the rainfall averaged 5.12 inches a few larvae survived up to 5 months. Although the number of larvae which were recovered from these two pastures beyond the third month is not very high, it does indicate that some of the larvae survived longer during the period of increased rainfall.

Although the above findings show that some of the larvae survived up to 5 months, the results also indicate that many of them perished during the first month. The average number of larvae recovered in the 4 trials after the first month of grass contamination was 6,572.5 (table 1) or only about 1.3 per cent of the ½ million larvae originally placed on the grass. The opinion that the survival of the majority of these larvae under natural conditions is comparatively short is in accord with other published reports. Goldberg and Rubin (1956) found that calves grazing on experimentally contaminated pasture plots acquired heavy infection with *C. punctata* 19 days after contamination and light infection 122 days after contamination. No infection was acquired 256 days after contamination. In another experiment carried out at a different time of the year, Goldberg and Lucker (1959) found that the calves acquired heavy cooperid infection 21 days after pasture contamination, and considerably lighter infection 63 days after contamination. No *C. punctata* were found in the calf placed on a plot 126 days after contamination.

The comparatively small number of adult cooperids which developed in the rabbits from the experimental feeding of larvae recovered from the contaminated grass (table 1) does not necessarily imply that only a small number of larvae fed were infectious. It has been previously pointed out by the writer (Alicata, 1958) that the rabbit is not an ideal host for this parasite and that ordinarily only a few of the parasites administered do become established. The rabbit, however, serves as a useful laboratory animal in establishing the infectiousness of at least some of the cooperid larvae.

Table 1. Number of larvae recovered from grass and surface soil at the indicated periods after contamination with 500,000 larvae of *C. punctata*. Figures in parentheses indicate the number of adult *C. punctata* from a rabbit to which these larvae were fed.

Pasture, Annual Rainfall	A. (Kawailoa Dairy), 36.80*		B. (Valley Dairy), 82.28**		Average
Average Monthly Rainfall Period	1.46 June-Nov., 1959	2.42 Jan.-June, 1060	3.85 June-Nov., 1959	5.12 Jan.-June, 1060	
1 month	7,600 (18)	5,650 (12)	5,550 (20)	7,540 (14)	6,572
2 months	280 (9)	350 (7)	700 (11)	560 (8)	472
3 months	45 (0)	70 (2)	105 (1)	362 (8)	145
4 months	0	5 (0)	25 (3)	60 (1)	22
5 months	0	0	0	5 (0)	1
6 months	0	0	0	0	0
Total	7,925	6,075	6,330	8,527	

*20 year average. **40 year average.

SUMMARY AND CONCLUSION

In each of 2 separate pastures on the Island of Oahu, having different intensity of rainfall, about ½ million infective *C. punctata* larvae were placed on grass plots, each isolated with a metal ring-band 7.5 inches in diameter. After 1 month an average of 1.3 per cent of the larvae originally placed on the grass was recovered and found infectious to rabbits. In the subsequent few months the larval recovery from the contaminated grass was sharply reduced. In one of the pastures which had an average monthly rainfall of 2.42 inches, some of the larvae survived up to 4 months. In another locality which had an average monthly rainfall of 5.12 inches, a few of the larvae survived up to 5 months. The present findings, together with those of other investigators, indicate that under natural conditions the majority of *C. punctata* larvae appear to perish during the first month, but with adequate moisture a few of them may survive up to several months.

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Helminth Infections of Healthy Florida Cattle, with a Note on *Cooperia spatulata*

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From August of 1957 through February of 1958, the writer had occasion to make parasitological examinations of 20 apparently healthy calves, 4 to 12 months old, which were shipped from farms in the northern two-thirds of Florida to Tifton, Georgia, for slaughter. The viscera were removed from the animals at a local abattoir and taken to the parasitology laboratory of the Animal Disease and Parasite Research Division at the Coastal Plain Experiment Station for postmortem examination. The technique used to recover the parasites was similar to that described by Porter (1942). Since the species recovered from these animals may interest other workers, the data are reported here.

Table 1 shows that 16 species of helminths representing 9 genera were recovered from the calves. However, not all species were encountered in every animal. The number of worms recovered ranged from 1,045 to 88,227 per animal; the average was 13,892. The smallest number occurred in a 9-month-old calf and consisted of five species: *Cooperia punctata* (50%),

Table 1. The Incidence and Numbers of Helminths Recovered, at Necropsy, from 20 calves from South-Central and Northwestern Florida.

	Calves Infected		Number of Worms	
	Number	Percent	Range	Average
Cooperia punctata	20	100	27-38,079	6,663
Haemonchus placei	17	85	1- 2,221	358
Ostertagia ostertagi	15	75	26- 2,561	586
Trichostrongylus axei	13	65	4- 7,440	1,664
Cooperia pectinata	13	65	3-12,653	1,932
Oesophagostomum radiatum	13	65	1- 537	84
Cooperia spp. (larvae and females)	11	55	80-52,000	6,900
Haemonchus similis	10	50	1- 182	53
Bunostomum phlebotomum	10	50	2- 153	41
Cooperia spatulata (males)	7	35	1- 520	123
Setaria cervi	5	25	1- 4	2
Trichuris discolor	4	20	1- 10	4
Cooperia oncophora	3	15	52- 2,800	1,204
Ostertagia lyrata	2	10	1- 1	1
Trichostrongylus longispicularis (males)	2	10	1- 12	6
Haemonchus spp. (larvae)	2	10	120- 338	229
Cooperia mcmasteri (male)	1	5	1- 1	1
Gongylonema pulchrum	1	5	3- 3	3

Haemonchus similis, Ostertagia ostertagi, and *Trichostrongylus axei* (each, 16%), and *H. placei* (2%). The latter were identified by spicule measurements (Roberts et al., 1954). The largest number of parasites was recovered from a 7-month-old calf and consisted of 11 species: *Cooperia* spp. (*C. punctata, C. pectinata, C. spatulata,* and unidentifiable *Cooperia* females and larvae) (90%), *T. axei* (8%), and *H. placei, H. similis, O. ostertagi, Bunostomum phlebotomum,* and *Oesophagostomum radiatum* (together, 2%). The calf having the number of parasites closest to the average harbored an infection very similar to the last-mentioned animal. According to Table 1, *C. punctata* had the highest incidence and *Gongylonema pulchrum* and *C. mcmasteri,* the lowest. The latter was reported from Florida cattle for the first time by Allen and Becklund (1958).

Two species, hitherto unreported from Florida and, until recently, rarely encountered helminths, namely, *C. spatulata* and *Ostertagia lyrata,* were recovered from 7 and 2 calves, respectively. Since *C. spatulata* was only recently reported for the first time from cattle in this country by the writer (1958), the recovery of this species from calves originating on farms scattered over south-central and northwestern Florida indicate that it is apparently well established in that State.

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A study of the Parasitic Habit of *Paratylenchus projectus* and *P. dianthus**

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Paratylenchus spp. are primarily ectoparasites of roots. Although some early workers found individuals of this genus in the cortex (Bally and Reydon, 1931) and in root lesions (Steiner, 1924), Goodey (1934) determined that they occurred almost entirely on the surface among the root hairs. He found one nematode still attached by its stylet to a processed root.

Linford, *et al.* (1949) published a comprehensive paper on the biology of *P. minutus,* giving particular emphasis to its feeding habits on roots growing in soil.

Observations presented in this paper were made on *Paratylenchus projectus* Jenkins, 1956, and *P. dianthus* Jenkins and Taylor, 1956, parasitizing seedling roots growing in agar.

MATERIALS AND METHODS

The feeding habit of *Paratylenchus projectus* was studied in detail in these investigations. That of *P. dianthus* was studied less thoroughly; therefore, reports given here concern *P. projectus* except where *P. dianthus* is mentioned specifically.

In preparation for observation of the feeding process in agar, seeds of red clover (Kenland variety), Ladino clover, and *Nicotiana alata* var. *grandiflora* Link and Otto (Jasmine Tobacco) were disinfested by wetting briefly with 95% ethyl alcohol and then placing them in an 0.35% sodium hypochlorite solution for 5 minutes. At the end of this time the solution was poured off and the seed rinsed in sterile water. The seeds were then plunged individually, with sterilized forceps, into 1½% water agar that had just solidified. Red and Ladino clover seedlings were grown in Petri plates containing approximately 25 ml of agar. The tobacco was grown both in Petri plates and in small cells made by cementing 2½ cm diameter glass rings with Canada balsam to 46 x 60 mm No. 2 cover glasses. The assembled cells were placed in Petri plates, each of which contained a piece of No. 2 filter paper, and sterilized in an oven. The heat dried the balsam, making a firm bond between the 2 parts. Approximately 2 ml of melted agar was placed in each of these cells before planting. The filter paper in the bottom of the Petri plates was kept moist with sterile water and served to retard drying of the agar.

Red and Ladino clover seed produced a primary root about ½ inch long in 3 days, at which time the nematodes were introduced into the dishes. Tobacco was somewhat slower in germination and growth, therefore, nematodes were not introduced until after 2-3 weeks.

Roots of the seedlings grew along the bottom of the containers, thus making them suitable for inversion onto the stage of the microscope for observation of nematode feeding. All seedlings were grown under fluorescent lights controlled by a time switch set for 12 hours illumination each day.

Infestation with nematodes was accomplished by two methods. Nematodes were obtained by leaching stock culture pots and were further cleaned by passage through an extraction pad. For short term observation, large numbers of nematodes were picked up with a small pipette and placed in a

*This paper is based chiefly upon part of a thesis submitted by the senior author in partial fulfillment of requirements for the Ph.D. degree in Plant Pathology from the Graduate College, University of Illinois.

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droplet of water on the agar a short distance from the growing root. As the water evaporated or was absorbed by the agar, the nematodes entered the agar and apparently were attracted to the growing roots. Plates infested in this manner usually became so contaminated with bacteria that they were of little value after about one week. However, in a few cases microphagus nematodes of the genus *Cephalobus* were accidentally introduced with the paratylenchs and proves quite beneficial by feeding on the bacteria and multiplying. Several of the small cells were then intentionally infested with a few of these cephalobids along with the paratylenchs, and it was in these cells that most of the satisfactory observations on feeding were made. A few such cells remained for study for 6-8 weeks.

For longer periods of observation, 1 to 10 freshly molted females with the old cuticle still intact, were passed through 3 successive changes of sterile water, allowing 2-3 minutes in each change. The nematodes were then transferred to a drop of sterile water on the agar using a nylon needle. This needle was first sterilized by placing it for 5 minutes in an 0.35% sodium hypochlorite solution, and was dipped into it briefly between transfers. Most cultures handled in this way remained free of bacterial growth for a long period. There were a few fungus contaminants but they grew sparsely and had no apparent influence on the results. One particularly good culture, started from a single female nematode on red clover in a Petri plate, was kept for 80 days by occasionally adding sterile water to the surface of the agar.

A 40X water-immersion objective with a free working distance of 1.9 mm was used to observe nematode feeding in the Petri plates. The coverslip used on small cells made possible the use of t 90X oil immersion objective.

Observations

Paratylenchus projectus and *P. dianthus* were both attracted to the young mature zone of roots growing in agar where they fed ectoparasitically much

Fig. 1. Female *Paratylenchus projectus* feeding on epidermal cell of tobacco root growing in agar. Note the deep insertion of the stylet with the dome of granular material around the tip. One egg has been laid.

as Linford, *et al.* (1949) reported for *P. minutus*. Females characteristically fed by inserting the stylet into epidermal cells (Fig. 1 and 2) or at the base of root hairs, but they were never observed feeding far out on the latter. Young larvae fed both on epidermal cells and root hairs, and frequently at some distance from the base of the latter (Fig. 3 and 4). The preadult larval stage of both species and the males of *P. dianthus* moved about the roots but made no attempt to feed.

The process of puncturing cell walls of tobacco and red clover by larvae and females was observed several times. It is a slow process requiring from 5 to 40 minutes. The nematode places its head against the cell wall and sets up a characteristic series of 4-16 relatively slow stylet thrusts. At the end of each series of thrusts, a rest period of 3-5 seconds ensues, then another series begins. As penetration of the cell progresses and the stylet becomes more deeply inserted, the series of thrusts becomes much longer with longer intervening rest periods. Counts of 53 to over 200 thrusts in a series, be-

Fig. 2. Female *Paratylenchus projectus* among root hairs of red clover growing in agar. It is feeding on an epidermal cell, has laid 2 eggs, and another is present in the uterus.

tween rest periods of 16-28 seconds, were recorded near the end of the penetration process for one particular individual.

After the wall had been penetrated and the stylet tip thrust well into the host protoplast, there was always a period of inactivity of some duration (55 minutes to 1 hour and 47 minutes) before pulsation of the median bulb began.

Nematodes feeding on the exceedingly thin and translucent roots of tobacco growing against the cover slip in the small cells could be seen to good advantage and with sharp resolution with a 90X oil-immersion objective lens. The nematode esophagus and the host cell protoplast were observed closely both before and during the process of feeding. In favorably situated individuals, saliva could be distinguished by its finely granular appearance throughout the entire length of the dorsal duct from the basal bulb to the duct opening into the lumen of the esophagus.

After the stylet tip was inserted into a cell and before pulsation of the median bulb began, saliva was seen to flow forward from the dorsal side of the basal bulb, through a slender passage along the dorsal side of the isthmus of the esophagus, through the broader duct that arches dorsally around the valve plates, and into the ampulla adjacent to the opening into the lumen of the esophagus behind the spear base. Commonly, the duct was inconspicuous when the stylet was first inserted, and it characteristically became more readily visible and distended as saliva accumulated, especially within the limits of the median bulb and anterior from that. Only after the duct became filled did pulsation of the median bulb begin. At no time was saliva seen to pass through the stylet or into the host cell, yet just before pulsation of the bulb began, a dome of granular matter was seen to form over the stylet tip. Soon after this pulsation began, the duct became narrower and less opaque, indicating that saliva had been moving out of it.

Fig. 3. *Paratylenchus projectus* larva feeding on a root hair of red clover growing in agar. Granular contents of protoplast are visible on either side of the stylet.

Fig. 4. *P. projectus* larva feeding on opposite side of same root hair as in Fig. 3 one day later.

Activity of the median bulb started gradually and was not limited to the characteristic pulsation that soon followed. The valve plates occasionally were seen to be pulled apart and then closed slowly. Also, sometimes there were muscular contractions that altered the shape of the bulb without opening the valve. Soon after the first activity of the bulb began, a rhythmic pulsation became established with the valve opening wide and closing with each beat. This was readily observable even in the smallest larvae. The rate of pulsation varied between individuals from about 100 to 180 per minute, but seemed relatively constant in one individual until the nematode was nearly ready to stop feeding. It then slowed gradually and stopped, after which the stylet was retracted.

Periods of continuous pulsation were extremely long for females on tobacco, lasting from one hour to 3-4 days. On red clover the period was even longer, often more than a week. One female was observed at 20 minute intervals during 7 hours, and many others were frequently observed after feeding started, without ever seeing an interruption of the pulsation until the nematode was ready to retract its stylet. However, some brief periods of interruption may have been missed. The feeding larva shown in Fig. 3 and 4, for example, was found feeding from the same root hair on two successive days, yet between observations it obviously had withdrawn its stylet and moved to the opposite side of the root hair to resume feeding. These observations are contrary to those of Linford, *et al.* (1949) for *P. minutus,* for they reported frequent interruptions and resumptions of pulsation during each prolonged period of feeding.

Rhythmic pulsation of the median bulb characteristically was of such vigor that it moved other parts of the esophagus in rhythm with it, and even caused pulsation of the granular matter over the stylet tip inside the host cell. Saliva throughout the entire length of the duct was seen to oscillate forward and backward. This was true especially in the narrow duct within the isthmus, where the oscillation was of wide amplitude making it impossible to estimate whether there was more movement forward than backward. At times there appeared to be some movement in the basal bulb independent of that imparted to it by the action of the median bulb, but this could not be certainly determined.

The epidermal cells of tobacco and root hairs of red clover were very favorable for observation of the host cell contents during feeding, but epidermal cells of red and Ladino clover were too opaque. As soon as pulsation of the median bulb began, the dome of granular material enclosing the stylet tip beat synchronously. It usually grew somewhat in size and sometimes appeared to occupy nearly half of the cell contents, but seemed always confined to only one cell. In root hairs it commonly occupied a considerable portion of the protoplast. Many of the granules became large oval bodies resembling yeast cells. In addition to the granular material, dark filaments frequently were seen in the cytoplasm and often led into the dome toward the stylet tip.

Cytoplasmic streaming and migration of the nucleus within the cell continued in apparently normal fashion during feeding, with the granular dome and stylet tip serving only as a minor barrier. After the stylet was retracted, the granules remained in somewhat the original position in tobacco epdermal cells. Even after 2 weeks these were still observable with cytoplasm continuing to stream. However, the protoplasts of red clover root hairs contracted and disappeared after being fed upon several days by larvae, leaving them apparently devoid of protoplasm.

Paratylenchus projectus and *P. dianthus* were relatively sedentary after feeding began. The location of certain females was marked and observed frequently while feeding on red clover roots. They consistently fed for several days at one site. When females were first put into agar containing a young clover seedling, they characteristically moved to the young mature region where they began feeding in 2-3 days. Several days later, after the growing point of the root had advanced some distance, they retracted the stylet and moved up to the young mature region where they began feeding again. A single female placed in a Petri plate fed at four different sites in this fashion during a period of 28 days and laid a cluster of eggs at each feeding site.

Only 2 females were discovered feeding as endoparasites in agar cultures. Both were located within epidermal cells of a secondary root of tobacco at its junction with the primary root and were feeding with their stylets inserted into adjacent epidermal cells. When roots from soil cultures were stained, cleared, and examined, however, both larvae and adults were found within epidermal cells or located either within or between cells of the cortex. These always were few in proportion to those that were present in the rhizosphere and appeared to have entered through wounds caused by other agencies. One of the most frequent portals of entry was through wounds made by emerging lateral roots. Senescent roots with cracked and deteriorating surfaces contained more nematodes than younger roots.

SUMMARY

A study was made of the parasitic relationships of *Paratylenchus projectus*, and *P. dianthus*. Feeding, observed on roots of seedlings growing in agar, was found to be chiefly ectoparasitic on epidermal cells and root hairs in the young mature region. Stylet insertion was a slow process requiring several minutes, followed by a period of relative inactivity in which saliva flowed forward in and filled the salivary duct and ampulla. No flow of saliva from the stylet was observed, but the salivary reservoir became less opaque and a granular dome built up around the stylet tip inside the host cell during feeding. The nature of this granular mass was not determined. Apparently it caused little disturbance to the host cell protoplast, as streaming continued in seemingly normal fashion during feeding and in epidermal cells of tobacco for many days after feeding had stopped and the stylet had been retracted. The protoplasts of red clover root hairs contracted and disappeared after prolonged feeding. No other evidence of local pathology was observed.

These paratylenchs were relatively sedentary once they began to feed in a suitable cell; certain larvae and females were observed to feed from a few days to over a week from one cell.

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Studies on Digenetic Trematodes of Hawaiian Fishes: Family Haplosplanchnidae*

MARY HANSON PRITCHARD and H. W. MANTER

The haplosplanchnids have a single intestinal cecum, a single testis, a Y-shaped excretory vesicle, and lack a cirrus or cirrus sac. Eggs in the uterus may or may not contain occulate miracidia; but if they do, the condition is correlated with greatly reduced vitellaria and presence in a *Mugil* sp. host, as noted by Manter (1957).

Skrjabin and Guschanskaja (1955) restricted the genus *Haplosplanchnus* Looss, 1902 to the latter group of species and named for it the subfamily Haplosplanchninae. Their key stressed not only the weakly developed vitellaria but the presence of a ventral peduncle and a vas deferens functioning as a seminal vesicle. The majority of the species were placed in *Schikhobalotrema* Skr. & Gusch., 1955, subfamily Schikhobalotrematinae (well developed vitellaria, no peduncle, and a well developed seminal vesicle).

The same authors considered *Laruea* Srivastava, 1939 a synonym of *Haplosplanchnus*. The peduncle is apparently larger than in *H. pachysomus* (Eysenhardt, 1829) Looss, 1902 and the posterior end of the body is described as "semi-spiral," but the large peduncle as well as the long posttesticular space appear to us to be specific characters. Although Yamaguti (1958) retains the genus *Laruea*, we agree with Skrjabin and Guschanskaja and consider it a synonym of *Haplosplanchnus*.

If two subfamilies are to be recognized here, the distinguishing characters should be embryonated or nonembryonated uterine eggs, the extent of the vitellaria, and, perhaps, the host (mullet or non-mullet). Certainly the peduncle of *H. purii* Srivastava, 1939 is little or no more prominent than those that may be found among the Schikhobalotrematinae, while the development of the seminal vesicle varies in both subfamilies. Vitellaria are definitely more extensive in *Schikhobalotrema*, at least extending backward beyond the testis and usually, but not necessarily, anterior to the acetabulum. The eggs of *Schikhobalotrema* contain very young embryos without eyespots even near the genital pore. This condition is in contrast with the oculate miracidia which occur throughout the uterus in the species of *Haplosplanchnus*. These seem to us to be more generic than subfamily characteristics.

Some of the following trematodes were collected in 1949 by one of us (M.H.P.); others, by Hilda L. Ching in 1959. The fishes were identified by Dr. William A. Gosline of the University of Hawaii.

Measurements of eggs are in microns; other measurements are in millimeters.

Schikhobalotrema robustum, n. sp. (Figs. 1-2)

HOSTS: *Pomacentrus jenkinsi* Jordan & Evermann, type host (Pomacentridae, damselfishes); 6 specimens from 1 or 2 of 30 hosts.
Acanthurus sandvicensis (Streets), manini or convict tang (Acanthuridae, surgeonfishes); 3 specimens from 1 of 56 hosts.
Chaetodon fremblii Bennett (Chaetodontidae, butterfly fishes); 3 specimens from 1 of 17 hosts.
Zebrasoma flavescens (Bennett), lau'i-palā or yellow tang (Acanthuridae, surgeonfishes); 5 specimens from 1 of 10 hosts.

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Location: Intestine.

Holotype: U. S. Nat. Mus. Helminth. Coll., No. 39062.

Description (15 specimens measured, but only 3 flattened dorsoventrally): Body elongate, 1.240 to 2.747 long by 0.449 to 0.469 wide (0.295 to 0.817 thick) at acetabular level; forebody about 1/5 to 1/4 body length, tapered to oral sucker at anterior end; hindbody 0.838 to 1.789 long, posterior half tapered and bluntly pointed terminally. Oral sucker 0.181 to 0.194 wide (0.114 to 0.221 deep) by 0.112 to 0.214 long with or without noticeable median projection on ventral edge, dorsal wall somewhat thicker than ventral wall; acetabulum 0.201 to 0.256 wide (0.120 to 0.348 deep) by 0.131 to 0.402 long; sucker ratio, based on widths, 1:1.03 to 1.33 (based on lengths, 1:1.08 to 2.2). Prepharynx 0.011 to 0.047 long, pharynx sometimes pressed against oral sucker; pharynx 0.040 to 0.080 long by 0.080 to 0.094 wide (0.064 to 0.107 deep); esophagus as long as pharynx or longer; large gland cells 0.030 to 0.048 in diameter associated with digestive system in forebody, ducts of a few glands leading to prepharynx while most lead to esophagus and anterior part of cecum; cecum overlaps testis 1/3 or more.

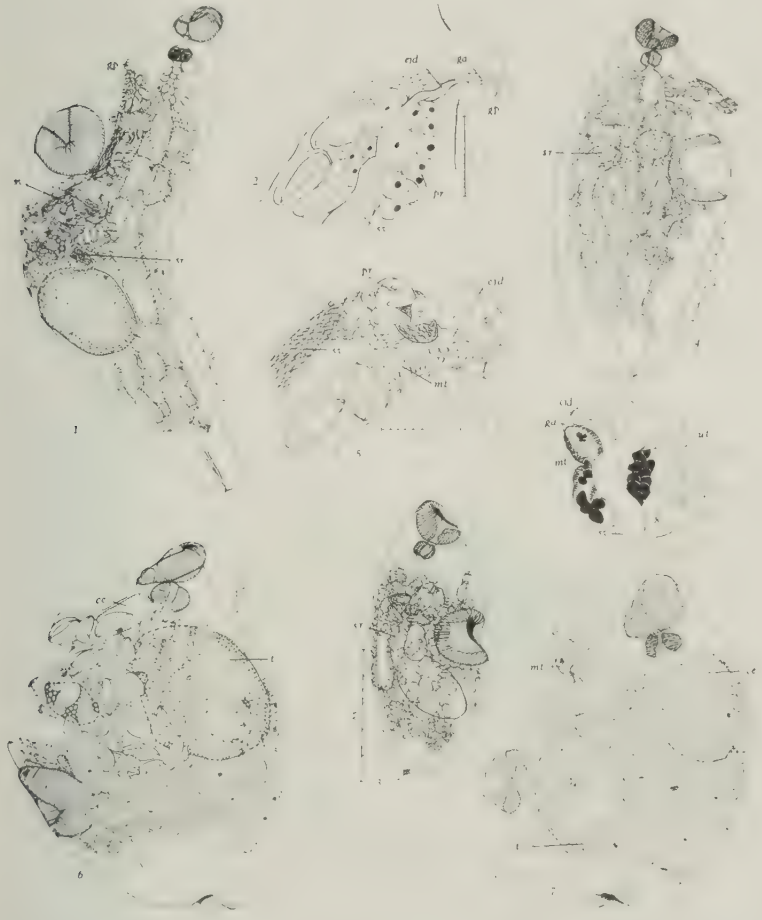
Testis rounded or oval, 0.332 to 0.594 long by 0.281 to 0.302 wide (0.141 to 0.415 deep), in middle or anterior part of hindbody 0.087 to 0.503 posterior to acetabulum, posttesticular space 0.344 to 0.938 long or longer than forebody; seminal vesicle long, tubular, sinuous with one or two distinct loops; not far from genital pore a constriction separates an anterior, tapering, carrot-shaped portion of seminal vesicle surrounded by large prostatic cells (Fig. 2); short, nonmuscular ejaculatory duct enters genital atrium; atrium shorter than terminal portion of seminal vesicle and thin-walled. Genital pore on a finger-like protrusion, midway between oral sucker and acetabulum, opposite esophagus.

Ovary subglobular, 0.096 to 0.194 long by 0.114 to 0.128 wide (0.074 to 0.168 deep), immediately pretesticular; seminal receptacle posterodorsal to ovary, spherical or ovoid, 0.040 to 0.228 long by 0.040 to 0.201 deep; vitellaria extending from level between genital pore and acetabulum to near posterior end of body, uninterrupted at least dorsally, masses large and elongate; uterus preovarian, looping once or twice near ovary and then extending directly to genital atrium; eggs few to numerous, uncollapsed eggs 72 to 83 long by 50 to 58 wide, collapsed eggs 64 to 98 by 30 to 56. Excretory pore terminal, excretory vesicle bifurcating near posterior end of testis, crura extending to anterior end of body.

Discussion: In size and long posttesticular space *S. robustum* is similar to *S. kyphosi* (Manter, 1947), but it differs markedly in having an unlobed ovary, much larger testis, larger sucker ratio, and less follicular vitellaria.

S. robustum is most similar to *S. girellae* (Manter and Van Cleave, 1951). It differs in being much larger, although the egg size is about the same. The difference in body size makes the eggs appear to be much larger in *S. girellae*. The so-called cirrus of *S. girellae* is probably a thick-walled tubular atrium although entrance of the uterus could not be seen in 15 paratypes. The atrial tube in *S. robustum* is thin-walled and shorter. Prostatic cells are much smaller in *S. girellae*.

Although the interruption of the vitelline glands opposite the testis was emphasized for *S. girellae*, we now find that indistinct strands of vitelline material connect the pre- and posttesticular vitelline masses in some of the paratype specimens. In *S. robustum* the dorsal connections are conspicuous.



All figures drawn (by M.H.P.) with the aid of a camera lucida; value of projected scale indicated in millimeters. Abbreviations used: ce, cecum; ejd, ejaculatory duct; ga, genital atrium; gp, genital pore; mt, metraterm; pr, prostatic cells; sr, seminal receptacle; sv, seminal vesicle; t, testis; ut, uterus.

Fig. 1. *Schikhobalotrema robustum* from *Pomacentrus jenkinsi*, holotype; lateral view (sinistral).

Fig. 2. *S. robustum*, holotype; lateral view (dextral) of terminal genital ducts.

Fig. 3. *Schikhobalotrema hawaiensis* from *Ctenochaetus strigosus*, holotype; lateral view.

Fig. 4. *Schikhobalotrema crassum* from *Pomacentrus jenkinsi*, holotype; lateral view.

Fig. 5. *S. crassum* from *P. jenkinsi*, paratype; lateral view of terminal genital ducts.

Fig. 6. *Schikhobalotrema glomerosum* from *Acanthurus sandvicensis*, syntype; lateral view.

Fig. 7. *S. glomerosum* from *Acanthurus achilles*, syntype; lateral view.

Fig. 8. *S. glomerosum* from *A. achilles*, syntype; lateral view of terminal genital ducts.

Schikhobalotrema hawaiensis, n. sp. (Fig. 3)

HOST: *Ctenochaetus strigosus* (Bennett), kole (Acanthuridae, surgeonfishes); 65 specimens from 5 of 19 hosts.

LOCATION: Intestine.

HOLOTYPE: U. S. Nat. Mus. Helminth. Coll., No. 39063.

DESCRIPTION (based on 18 specimens): Orange-red when alive; body plump, 0.938 to 1.441 long by 0.422 to 0.583 wide, widest at level of acetabulum; forebody only slightly shorter than hindbody, both ends tapered and bluntly rounded. Oral sucker 0.149 to 0.201 wide by 0.118 to 0.168 long, dorsal edge thicker than ventral edge; acetabulum at midbody or slightly anterior, 0.194 to 0.268 wide by 0.188 to 0.261 long, aperture rounded or somewhat transverse; sucker ratio 1:1.2 to 1.4. Prepharynx very short, pharynx usually contiguous with oral sucker; pharynx wider than long, 0.035 to 0.080 long by 0.056 to 0.101 wide; esophagus about as long as pharynx; cecum more or less dorsal, extending to testicular level, overlapping ⅓ to almost all of testis.

Testis oval or elongate-oval, 0.168 to 0.415 long by 0.127 to 0.268 wide, median in hindbody with anterior end near or overlapping posterior edge of acetabulum (in latter case, testis lies in dorsoventral plane with anterior end dorsal to acetabulum); seminal vesicle long, slender, sinuous or coiled, beginning at level of ovary; prostatic cells not well developed; no prostatic vesicle or muscular cirrus, although a pronounced constriction separates distal, carrot-shaped portion of seminal vesicle; genital atrium shallow and not conspicuously muscular; genital pore median, about midway between suckers.

Ovary elongate, sometimes slightly indented or bent near middle, slightly dextral between testis and acetabulum or dorsal to acetabulum, 0.066 to 0.154 long by 0.035 to 0.109 wide, overlapping testis or not; seminal receptacle lateral or dorsal to ovary, 0.074 to 0.104 in diameter, often inconspicuous; vitellaria extending dorsally and laterally between level of genital pore (occasionally posterior edge of pharynx) and posttesticular area, follicles often elongate. Parenchyma contains numerous, more or less conspicuous, large, nucleated cells. Uterus preovarian, containing few to as many as 30 eggs, joining genital atrium without forming metraterm; eggs yellowish, usually collapsed, 70 to 104 long by 32 to 58 wide (examples of only slightly dented eggs: 72 by 50, 75 by 53, 77 by 53, and 80 by 58). Excretory pore terminal; a short, narrow stem leading to excretory vesicle; vesicle extending to testis before bifurcating; crura extending forward to level of esophagus.

DISCUSSION: Three species, *S. brachyurum* (Manter, 1937), *S. pomacentri* (Manter, 1937), and *S. adbrachyurum* Siddiqi and Cable, 1960, have the acetabulum located at or slightly behind the midbody. Tortugas, Florida, is the type locality for the first two species and Puerto Rico is the type locality for *S. adbrachyurum*. Manter (1940) has also reported *S. pomacentri* from the Galapagos Islands.

S. hawaiensis is like *S. pomacentri* in that the hindbody may equal the forebody in length but is never shorter, and the anterior vitellaria do not extend to the oral sucker. *S. hawaiensis*, however, differs from *S. pomacentri* by lacking a prostatic vesicle, by lacking a posterior marginal projection on the oral sucker, by a less tapered and more rounded hindbody, in the testis which is larger than the acetabulum and immediately posterior to it (rather than being in the middle of the hindbody).

S. hawaiensis is like *S. brachyurum* in lacking the prostatic vesicle, in lacking a posterior marginal projection of the oral sucker, in the testis that may

overlap the acetabulum, and the seminal vesicle that is sinuous rather than almost straight. It differs from *S. brachyurum* in that the hindbody is never shorter than the forebody (though it may be nearly equal), the vitellaria usually extend only to the level of the genital pore (and no farther forward than the posterior edge of the pharynx), the uterus is entirely preovarian, and the acetabulum is slightly smaller affecting the sucker ratio (1:1.2 to 1.4 as compared with 1:1.5 to 2 for *S. brachyurum*).

S. adbrachyurum has the acetabulum slightly posterior to midbody, but the testis is near the posterior end of the body, the ovary is lobed, the seminal vesicle is shorter, the eggs are wider, and the body is smaller.

Schikhobalotrema crassum, n. sp. (Figs. 4-5)

HOST: *Pomacentrus jenkinsi* Jordan & Evermann (Pomacentridae, damselfishes); 5 specimens from 1 or 2 of 30 hosts.

LOCATION: Intestine.

HOLOTYPE: U. S. Nat. Mus. Helminth. Coll., No. 39066.

DESCRIPTION (based on 4 specimens, all side views): Body 1.675 to 1.916 long by 0.771 to 1.018 deep at acetabular level; forebody 0.335 to 0.670 long, ¼ to ⅓ body length; hindbody 0.804 to 1.052 long, tapered only slightly, terminal portion somewhat contracted; cuticula thick (0.010 to 0.022). Oral sucker subterminal, 0.147 to 0.214 long by 0.147 to 0.201 deep, dorsal wall somewhat thicker than ventral wall; acetabulum 0.315 to 0.415 long by 0.214 to 0.308 deep with rounded aperture; acetabulum about twice as large as oral sucker. Prepharynx short, 0.013 to 0.020 long; pharynx 0.074 to 0.087 long by 0.087 to 0.101 deep; esophagus very short; a few large gland cells associated with esophagus and possibly prepharynx; cecum extending to near posterior end of testis.

Testis rounded or oval, 0.436 to 0.536 long by 0.295 to 0.375 deep, overlapping acetabulum ⅓ or more, posttesticular space as long as forebody or slightly longer; seminal vesicle tubular, almost straight; terminal portion of seminal vesicle surrounded by well developed prostatic cells; short ejaculatory duct connecting with short genital atrium. Genital pore median, somewhat protuberant, midway between suckers.

Ovary rounded, pretesticular, dorsal to anterior half of acetabulum, 0.127 to 0.168 long by 0.114 to 0.174 deep; seminal receptacle posterior or posterodorsal to ovary, oval, 0.181 to 0.237 long by 0.107 to 0.147 wide; vitelline follicles both rounded and elongate, extending from level of pharynx (or genital pore) to near posterior end; uterus pretesticular but not entirely preovarian, rather short, usually coiling once; metraterm present (Fig. 5) but may be inconspicuous when extended by eggs; eggs collapsed, 75 to 94 by 40 to 48. Excretory pore probably terminal, although contraction of body makes it seem ventroterminal; excretory vesicle short, oval or rounded, bifurcating at posterior edge of vitellaria, crura not traced. The name *crassum* (=thick, stout) refers to the thick body.

DISCUSSION: *S. brachyurum* and *S. hawaiensis* may also have the testis near or even overlapping the acetabulum. *S. brachyurum* has conspicuously larger suckers relative to body size, although the sucker ratio is probably similar, the hindbody is much shorter, and the vitellaria are more extensive and more follicular. *S. hawaiensis* has a shorter hindbody, a smaller sucker ratio, lacks large prostatic cells, lacks a well-differentiated metraterm, and lacks the conspicuously thick cuticula.

S. crassum also resembles *S. robustum* with which it may be sympatric, but *S. crassum* has a thicker body with a conspicuously thick cuticula, the testis overlaps the acetabulum ⅓ or more with the ovary correspondingly more anterior, and the excretory vesicle is shorter.

Schikhobalotrema glomerosum, n. sp. (Figs. 6-8)

HOSTS: *Acanthurus sandvicensis* (Streets), manini or convict tang, type host (Acanthuridae, surgeonfishes); 1 specimen from 56 hosts.
A. achilles Shaw, paku'iku'i or Achilles tang; 1 from 2 hosts.

LOCATION: Intestine.

SYNTYPES: U. S. Nat. Mus. Helminth. Coll., Nos. 39064 & 39065.

DESCRIPTION (based on both specimens): Body subspherical, 0.784 to 0.838 long by 0.556 to 0.570 thick; forebody more than twice as long as hindbody, tapered slightly toward oral sucker; hindbody truncate with somewhat invaginated end. Oral sucker 0.128 to 0.147 deep by 0.087 to 0.128 long, dorsal wall thicker than ventral wall, one specimen with small median projection on ventral edge; acetabulum about ¾ body length from anterior end, 0.083 to 0.127 deep by 0.176 to 0.201 long; sucker ratio greater than 1:1. Prepharynx very short; pharynx 0.051 to 0.059 long by 0.072 to 0.088 deep, pressed against oral sucker; esophagus short; cecum extending to anterior level of acetabulum.

Testis elongate, oval or ovate, 0.328 to 0.362 long by 0.192 to 0.261 deep, posterodorsal to acetabulum; seminal vesicle tubular, sinuous; ejaculatory duct short, entering genital atrium; prostatic cells not observed. Genital pore median, at esophageal level, somewhat protuberant.

Ovary preacetabular and pretesticular, ovate, 0.099 to 0.112 long by 0.144 to 0.182 deep, seminal receptacle not observed; vitellaria diffuse, from level of pharynx to near posterior end of body, massed together or in elongate groups; uterus extending posteriorly to level of acetabulum and then forward, metraterm joining genital atrium; 23 eggs in one specimen, yellowish, 83 to 88 long by 56 to 67 wide (collapsed eggs 83 to 107 long by 43 to 59 wide). Excretory pore opening into funnel-shaped depression at posterior end of body; vesicle not traced forward. The name *glomerosum* (=like a ball, round) refers to the shape of the body.

DISCUSSION: Although one specimen appears to have been overly flattened (ruptured body wall, testis pushed anteriorly and dorsally with a corresponding displacement of the cecum ventrally) (Fig. 6), this specimen contains mature eggs and has normal suckers. The other specimen is more macerated and has abnormal eggs, but the organs seem to be normally situated, and the terminal genital ducts are easily observed (Figs. 7-8).

S. brachyurum (Manter, 1937) and *S. adbrachyurum* Siddiqi and Cable, 1960 are the only other species in which the acetabulum may be found posterior to the midbody. In both species the cecum extends posterior to the acetabulum, the acetabulum is larger, the ovary and testis are in the hindbody, the testis is smaller than the acetabulum, the vitellaria are follicular, and the body is more elongate.

Schikhobalotrema obtusum (Linton, 1910) Skr. & Gusch., 1955

HOSTS: *Acanthurus sandvicensis* (Streets), manini or convict tang (Acanthuridae, surgeonfishes); 7 specimens from 2 to 12 of 56 hosts.

A. achilles Shaw, paku'iku'i or Achilles tang (Acanthuridae); 7 specimens from 1 of 2 hosts.

A. sp. (Acanthuridae); 2 specimens

LOCATION: Intestine.

DISCUSSION: These specimens vary considerably. Some resemble Linton's (1910) Fig. 160 (corrected to show a single cecum) or Siddiqi and Cable's (1960) Fig. 18; some resemble Manter's (1937) Fig. 1 except that none possesses such an elongate testis; and a few even resemble *S. crassum* in the position of the testis. Some specimens show a conspicuous ventral projection of the oral sucker, others seem to lack the projection; some show 4 to 6 pairs of oral papillae, others lack papillae. The species, however, is distinguished by the almost equal size of the suckers, a hindbody noticeably longer than the forebody, the testis usually in middle of the hindbody, the lack of a prostatic vesicle and muscular genital atrium, and a rounded aperture of the acetabulum.

The type specimen of *S. obtusum* was kindly loaned by the U. S. National Museum. There are three specimens on the slide, No. 8518, two of which are broken and all of which are located at the extreme edge of the cover glass where the mounting medium causes a distorted view. The almost equal size of the suckers and the elongate hindbody may, however, be confirmed.

Manter (1955) reported this species from Hawaii, also from *Acanthurus sandvicensis*. In addition it has been reported from Tortugas, Florida, and Puerto Rico.

SUMMARY

Four new species of *Schikhobalotrema, S. robustum, S. hawaiensis, S. crassum,* and *S. glomerosum,* are described from shore fishes of Hawaii (Acanthuridae, Pomacentridae, and Chaetodontidae). *S. obtusum* (Linton, 1910) Skr. & Gusch., 1955 is reported from 2 species of Acanthuridae.

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Amended Descriptions of *Belonolaimus gracilis* Steiner, 1949 and *B. longicaudatus* Rau, 1958 (Nematoda: Tylenchida)*

GEORGE J. RAU**

The genus *Belonolaimus* was established with *B. gracilis* as the only species (Steiner, 1949). All populations of the genus were usually referred to this species until 1958, when Rau, on the basis of extensive collections, described *B. longicaudatus* and expressed the opinion that this species, not *B. gracilis,* is the common sting nematode in the southeastern United States. This opinion was based on the fact that collections made in the vicinity of Ocala, the type location for *B. gracilis,* and in many other parts of Florida did not contain nematodes corresponding to the description of this species. Since 1958, many additional collections of *Belonolaimus longicaudatus* have been made, and sting nematodes corresponding in most details to the description of *B. gracilis* have been found around the roots of long leaf pine (*Pinus palustris* Mill.) in the Ocala National Forest near Ocala, Florida. This is the reported type location, and the nematodes were successfully transferred to the reported type host, slash pine (*Pinus elliottii* Engelm. (*P. caribaea* Morelet)), growing only on Lakewood sand, in the greenhouse. Steiner also reported long leaf pine as a host of *B. gracilis.*

The purposes of this paper are to amend the original description of *B. gracilis,* record variation in this species, amend the description of *B. longicaudatus* by correction of some of the dimensions originally given, and present additional information on another collection from the type location. The specimens of *B. gracilis* used in this study have been deposited in the United States Department of Agriculture Nematode Collection, Nematology Investigations, Beltsville, Maryland.

Measurements in Table 1 include those from the original illustration and drawings of *B. gracilis* as well as of specimens collected December 12, 1958, near the type location. For *B. longicaudatus,* measurements from the original description are given as well as of a population collected September 24, 1958, from the type location. In addition, some useful ratios were calculated. These are the stylet length divided by the tail length (S/T) and an index number obtained by dividing the length anterior to the vulva (A) by the length posterior to the vulva (P) and then dividing the quotient by S/T.

The females of *B. gracilis* can usually be separated from those of *B. longicaudatus* by average stylet length since stylet lengths overlap only at the extremes of the ranges. The same is true of tail length or of tail length as related to width of the body at the anus. When the ratio of stylet length to tail length is calculated, there is no overlap of ratios.

The median bulb of *B. gracilis* is nearly spherical, while that of *B. longicaudatus* is elongated. Individuals of the latter species nearly always have an opposing pair of sclerotized pieces in the vagina which are lacking in *B. gracilis.* Also, in *B. longicaudatus* the tail is hemispherical with terminus 5.9 microns (4.2-7.8 microns) from the protoplasmic portion while that of *B. gracilis* is convex-conoid with the terminus 11.5 microns (8.4-15.4 microns) from the protoplasmic portion of the tail.

Ten female specimens of *Belonolaimus gracilis* were also found on a slide sent in by Simon Malo, formerly of the Lake Alfred Experiment Station,

*Cooperative investigations of the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, and the Central Florida Experiment Station.

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Florida. This slide was labeled "Hamlin Red grapefruit on lemon. Grove 304, Tavares, Florida."

As stated in the previous paper (Rau, 1958), *B. longicaudatus* is apparently the common sting nematode of Florida. Large numbers were found on the slides of nematodes from citrus groves sent in by Mr. Malo. *B. longicaudatus* was also found on the roots of slash pine at the Turpentine Experiment Station, Olustee, Florida. It was successfully transferred from sweet corn to slash pine seedlings growing in Leon sand. Along the seashore, *B. longicaudatus* is often found on various hosts, but particularly on sea oats (*Uniola paniculata* L.) growing in damp coarse white sand. Another undescribed species of *Belonolaimus* is found on this host growing in damp fine grey sand or sand containing large quantities of red coquina shell.

The ratios between stylet and tail lengths and index numbers of various

Table 1. Measurements of *Belonolaimus gracilis* and *B. longicaudatus*

	Belonolaimus gracilis		*Belonolaimus longicaudatus*	
	Steiner 1949(I)	Type Locality (II)	Rau, 1958 (III)	Type Locality (IV)
Female:				
Length (mm)	2.15	1.9 (1.4-2.3)	2.3 (2.0-2.6)	2.5 (2.0-3.0)
a	52	49 (39-63)	65 (56-75)	64 (55-74)
b*	6.1	6.7 (5.1-9.8)	8.4 (7.3-9.9)	9.0 (7.2-12.6)
c	19.2	23 (16-28)	16 (15-18)	18 (13-21)
Vulva	52%	53% (50-57)	50% (46-54)	49% (47-51)
Tail length (microns)	120	78 (53-134)	140 (117-163)	156 (115-183)
Stylet length (microns)	157	152 (130-168)	118 (100-133)	127 (116-140)
Phasmic-terminus (microns)	77	65 (49-83)	96 (79-109)**	96 (83-129)
Tail length				
Anal width	3.0	2.6 (1.8-3.6)	4.4 (3.5-5.0)***	4.7 (3.8-6.0)
Stylet length / Tail length (S/T)	1.31	1.76 (1.33-2.31)	.84 (.68-1.00)	.82 (.67-1.14)
Index number	.82	.64 (.52-.89)	1.17 (.96-1.40)	1.18 (.88-1.41)
Male:				
Length (mm)	1.7	1.9 (1.4-2.5)	2.3 (2.0-2.6)	2.5 (2.0-3.0)
a	52	52 (44-61)	64 (54-76)	64 (55-74)
b		6.3 (5.1-7.2)	7.1 (6.3-8.1)	7.5 (7.0-8.1)
c	14.7	17 (13-29)	15 (13-17)	15 (13-17)
Tail length # (microns)	116 †	99 (60.2-140.0)	127 (100-150)	141 (127-157)
Stylet length (microns)		137 (99-154)	113 (107-123)	120 (111-132)
Stylet length / Tail length (S/T)#		1.37 (1.07-1.96)	.95 (.76-1.19)	.85 (.76-.97)
Phasmic-terminus (microns)	67	71 (34-95)	94 (83-102)**	94 (74-129)
Spicule length § (microns)	51	45 (35-50)	44 (43-46)	43 (38-49)
Gubernaculum (microns)	20	16 (14-18)	16 (15-17)	17 (15-18)

(I) From the original description and illustrations of Steiner, 1949. There is probably an error in the stated magnification of the drawing of the male tail (Fig. 32B). By calculation from the reported length and gamma value, the tail of the male is 116 microns long. If this is correct, the magnification of the drawing is 586 and not 450.

(II) 61 females and 58 males from a population collected near type location at Ocala, Florida, December 12, 1958.

(III) 22 females and 22 males described by Rau, 1958, including corrections.

(IV) 31 females and 28 males from a population collected at the type location, Sanford, Florida, September 24, 1958.

* The length of the esophagus was measured to the posterior end of the glandular lobe.

**In the original description these figures were reported as 126 microns (104-144) for females and 98 microns (83-131) for males, because of improper calibration of the micrometer used.

*** Some confusion seems to have resulted from the failure of the author to indicate the exact position of the anus in Figure 1C (Rau, 1958). This is located at the small irregularity of the body contour on the left side of the drawing. This drawing, being on a much larger scale, gives a better idea of the average proportion of tail length to anal body diameter than Figure 1E, which was intended only to indicate the general size and shape of the body.

Tail length of the male was measured along the chord, that is, along the straight line from anus to terminus, except for the measurement in the first column, which was made from a drawing of a ventral view.

§ Spicule length was measured along the chord.

† The measurement was made from a ventral view, not along the chord.

populations of *Belonolaimus longicaudatus* and the undescribed species of *Belonolaimus* apparently indicate that there are many ecotypes which can be correlated with the environmental conditions. A great majority of the populations of *B. longicaudatus* have lip regions set off from the head by deep constrictions. Populations agreeing with the one from the type location in all other respects have been found with distinctly less marked constrictions which is characteristic for the undescribed species. These have been collected from beans in West Palm Beach County, Florida, sea oats at Cape Blas, Florida, and Padre Island, Texas. In addition, a large population received from W. Birchfield from an unknown host at Grand Island, Louisiana, exhibited similar characteristic but in degrees varying from slight to very slight constricted lip regions. A population of *Belonolaimus* sent by L. Miller had convex conoid tails with terminus 7.7 microns (5.6-9.8 microns) from the protoplasmic portion of the tail. Data received from L. Miller showed several populations of *Belonolaimus* from Holland, Virginia with stylet lengths longer than those generally found in this species. Also populations of *Belonolaimus* from sea oats, Padre Island, Texas, and peanuts, Margaretsville, North Carolina, sent by J. Sasser did not show the opposing sclerotized pieces in the vagina which are characteristic for the populations of *B. longicaudatus*.

The above variant populations apparently belong to the same species—*B. longicaudatus*—and also fall within the limits of the S/T and the A/P ÷ S/T indexes. The various characters of these populations probably provide a basis for the study of adaptability to specific environments, but this has not as yet been established.

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Macrostomtrema tamsuiensis, n. gen., n. sp. (Trematoda: Microphallidae) from river crabs of Taiwan (Formosa)

JUI-KUANG CHIU

During a study on trematodes for which river crabs are the intermediate host, an undescribed species of metacercaria was recovered from two species of crabs, *Sesarma bidens* and *Sesarma plicatum*, collected from the Tamsui River in northern Taiwan. After experimental feedings of the metacercariae to mammals and birds, adult flukes were obtained from the small intestine of ducklings. Careful observations of the flukes showed them to represent a new genus and a new species in the family *Microphallidae*. Hence a new genus *Macrostomtrema* is established and the species is named as *Macrostomtrema tamsuiensis*, n. gen., n. sp.

MATERIALS AND METHODS

During the period from October 1959 to August 1960, six species of river

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crabs were captured from the Tamsui River, 4 to 8 kilometers above its mouth. These included 212 *Helice tridens tridens* (de Haan), 80 *Helice tridens wuana* Rathbun, 134 *Chasmagnathus convexus* de Haan, 156 *Sesarma* (*Holomethopsus*) dehaani Edwards, 405 *Sesarma* (*Chiromantes*) *bidens* (de Haan) and 523 *Sesarma* (*Parasesarma*) *plicatum* (Latreille). Only internal organs of these crabs, such as gills, liver, genital glands and heart etc., were picked out from the crabs and compressed between two large slides and then examined for metacercariae under dissecting microscope. The metacercaria of this fluke was found only in the latter two of the six species of crabs examined.

The metacercariae were fed to mice, rats, chicken and ducklings. Fecal examinations of the experimental animals were carried out every day. The animals were sacrificed at different intervals after feeding and were examined for adult trematodes. The morphology was studied in living as well as stained specimens. Some of the flukes were stained in toto with Delafield's hematoxylin or carmine after fixing in 60% alcohol. About 50 worms were embedded in paraffin, serially sectioned and stained with hematoxylin and eosin.

All measurements of the parasite were made on adult worms or excysted larvae fixed in 2% formalin solution, under slight cover glass pressure. The drawings were made with the aid of a camera lucida.

FAMILY MICROPHALLIDAE TRAVASSOS, 1920

Macrostomtrema, n. gen. (Plate II, Figs. 1, 2)

GENERIC DIAGNOSIS: A minute, pyriform or fusiform trematode, entire body covered with spines. Oral sucker subterminal, large; prepharynx short; pharynx elliptical, well developed; esophagus slender; intestinal ceca short, stumpy, thick-walled, terminating in lateral fields of acetabulum. Acetabulum much smaller than oral sucker, in equatorial region, surrounded by cirrus sac. Testes round or ovoid, more or less diagonally placed, near posterior extremity. Cirrus sac large, ring-shaped, encircling acetabulum, containing a large, tubular seminal vesicle and well developed prostate glands, with tubular, muscular accessory organ between ends. Genital atrium complex, opening dextral to acetabulum. Ovary ovoid or spherical, sinistral to cirrus sac. Both seminal receptacle and Laurer's canal absent. Vitelline follicles situated in front of each testis in cluster. Uterus winding in posterior part of body, containing numerous small eggs. Excretory bladder pyriform, extending forward to level of testes. Intestinal parasite of bird, experimentally.

TYPE SPECIES: *Macrostomtrema tamsuiensis*, n. sp.

DISCUSSION: It is evident that this fluke belongs to the family *Microphallidae* Travassos, 1920, because of its characters: minute body, short ceca, presence of cirrus sac and absence of seminal receptacle. According to Yamaguti (1958), there are five subfamilies under the family *Microphallidae*, i.e., *Microphallinae* Ward, 1901; *Gymnophallinae* Odhner, 1905; *Maritrematinae* Belopolskaia, 1952; *Parvatrematinae* Yamaguti, 1958 and *Pseudosellacotylinae* Yamaguti, 1958. In having a well developed cirrus sac the present fluke is placed in the subfamily *Maritrematinae*, since no cirrus sac can be observed in the members of other subfamilies.

The subfamily *Maritrematinae* includes the genera: *Maritrema* Nicoll, 1907; *Odhneria* Travassos, 1921; *Microphalloides* Yoshida, 1938; *Gynaecotyla* Yamaguti, 1939; *Maritreminoides* Rankin, 1939; *Pseudospelotrema* Yamaguti, 1939; *Pseudospelotrematoides* (Yamaguti, 1939); *Diacetabulum* Belopolskaia, 1952; *Numeniotrema* Belopolskaia, 1952; *Pseudomaritrema* Belo-

polskaia, 1952 and *Probolocoryphe* Otagaki, 1958. The present fluke is similar to the members of genera *Maritrema* and *Microphalloides* in having a well developed cirrus sac and complex genital atrium. But in the members of genus *Maritrema,* vitelline follicles are arranged in a complete or incomplete ring, and the cirrus sac with simple extremities is more or less curved posteriorly and lies transversely or obliquely behind cecal arch (Coil, 1955; Yamaguti, 1958). In the species of genus *Microphalloides,* vitellaria occupy a small area on the antero-lateral aspect of each intestinal cecum, and the cirrus sac is large, semicircular in shape, lying transversely with its concave side posterior in the central portion of body, each of the distal ends of the sac bearing a chitinous process of different shape (Yoshida, 1938; Miyazaki, 1938; Chiu, 1960). The present fluke differs from other members of the two genera in the following respects: (1) vitelline follicles arranged in a cluster in front of each testis; (2) cirrus sac large, well developed, ring-form, encircling acetabulum, with genital accessory organ between distal ends. By these characters this fluke can be differentiated from the members of other genera.

Macrostomtrema tamsuiensis, n. sp. (Plate II, Figs. 1-3)

DIAGNOSIS OF SPECIES: (all measurements in millimeters with average given in parentheses) With characters of the genus. In living specimens, minute trematode actively motile, changing its shape and size continuously with pointed posterior extremity. Posterior portion of body light brown in color owing to uterine eggs.

Based on 20 specimens fixed in 2% formalin; a minute, pyriform or fusiform fluke, entire body covered with spines, 0.5402 to 0.6891 long and 0.3110 to 0.3927 wide at acetabulo-vitellaria region (0.5986 x 0.3694). Oral sucker large, 0.0990 to 0.1337 long and 0.1188 to 0.1419 wide (0.1175 x 0.1313); acetabulum near equatorial region, much smaller than oral sucker, 0.0726 to 0.1056 long and 0.0719 to 0.0950 wide (0.0825 x 0.0838); prepharynx very short, 0.0228 to 0.0257 long (0.0241); pharynx elliptical, 0.0429 to 0.0561 long and 0.0271 to 0.0383 wide (0.0502 x 0.0343); esophagus slender, 0.0604 to 0.0750 long (0.0653); intestinal ceca short, stumpy, thick-walled, 0.1679 to 0.1825 long (left, 0.1723; right, 0.1767). Testes somewhat obliquely placed in posterior part of body, right testis always situated slightly anterior to left one, round or ovoid, 0.1168 to 0.1372 in transverse diameter by 0.0993 to 0.1168 longitudinally (0.1285 x 0.1037), both testes about same size. Vas efferens from each testis running antero-medially, fused posterior to left extremity of cirrus sac to form vas deferens which leads to cirrus sac. Cirrus sac large, well developed, ring-form, encircling acetabulum, containing a long, tubular seminal vesicle filled with spermatozoa, well developed prostate glands, an ejaculatory duct and an inconspicuous cirrus organ. Cirrus sac always with its maximum width in region of prostate glands, 0.0394 to 0.0438 (0.0423). Left extremity of sac bearing tubular genital accessory organ provided with conspicuous, chitinous end connected with right hand part of sac by muscle fibers at bottom and forming complete ring. Whole cirrus sac ring measuring 0.1460 to 0.1650 in longitudinal diameter by 0.1679 to 0.1956 in transverse (0.1504 x 0.1883). Complex genital atrium situated in dextral vicinity of acetabulum, bearing two chitinous openings. Cirrus organ opening into lower of two openings. Upper appears to be opening of uterus. Ovary lies sinistral, in region bounded by cirrus sac, distal end of left intestinal cecum and left vitellaria, ovoidal or spherical in shape, 0.0861 to 0.1022 long and 0.0686 to 0.0891 wide (0.0949 x 0.0773). Oviduct running postero-medially from ovary to oötype located behind acetabulum and surrounded by

Mehlis' glands. Vitellaria arranged in cluster of 7 follicles in front of each testis. Vitelline ducts, conspicuous, running transversely from vitellaria and fusing at median line to form a common vitelline duct. Neither seminal receptacle nor Laurer's canal can be observed. Uterus filled with eggs, coiling at hindbody and extending forward to genital atrium. Uterine eggs operculate, ovoid in shape, light yellow-brown in color and containing a miracidium. Measurements of 40 eggs were 0.0165 to 0.0195 long by 0.0102 to 0.0129 wide (0.0173 x 0.0115). Excretory bladder pyriform, with paired collecting tubules at its apex. Flame cell pattern is 2(1+2+2+1+1).

Plate I

Fig. 1. *Sesarma (Chiromantes) bidens* (de Haan).
Fig. 2. *Sesarma (Parasesarma) plicatum* (Latreille).

EXPERIMENTAL HOSTS: Moscovy duck, *Cairina moschata* (Linnaeus) and mule duck (*Cairina moschata* x *Anas platyrhyncha* var. *domestica*).

HABITAT: Small intestine.

TYPE LOCALITY: Chu-wei and Kuan-tu villages, along the Tamsui River, Taipei County, Taiwan (Formosa).

INTERMEDIATE HOST: Brackish water crabs, *Sesarma* (*Chiromantes*) *bidens* (de Haan) and *Sesarma* (*Parasesarma*) *plicatum* (Latreille).

TYPE SPECIMEN: in parasite collections, Department of Parasitology, College of Medicine, National Taiwan University, Taipei, Taiwan (Formosa) and paratypes in the Helminthological Collections of the U.S.N.M. No. 39481.

METACERCARIA OF *Macrostomtrema tamsuiensis* (Plate II, Figs. 4, 5)

Out of six species of the crabs examined, only two species, *Sesarma bidens* and *Sesarma plicatum*, were infected with metacercariae of the fluke. The incidence of infection was very high as shown in the following table.

Incidence of metacercariae of *M. tamsuiensis* in the crabs

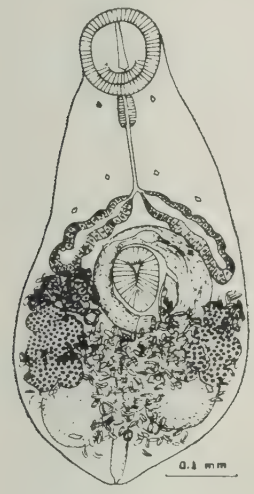
Species of crab		Number examined	Number infected	Percent infected	Size of crab (Breadth of carapace)
Sesarma bidens	M	288	215	74.7	10 — 25 mm
	F	117	87	74.4	
	T.	405	302	74.6	
Sesarma plicatum	M	406	329	81.0	12 — 28 mm
	F	117	94	80.3	
	T.	523	423	80.9	

The metacercaria was found only in the glandular masses lying under the rostella of the crab and varied from 1 to about 300 per crab. In heavy infections, mostly in large crabs, the metacercariae occupy the whole glandular mass. The location and numbers of metacercariae are similar in both sexes of the two species of crabs.

Metacercaria (based on 20 living specimens) is elliptical in shape, 0.3679 to 0.4351 long by 0.2044 to 0.2367 wide (0.4088 x 0.2175) and cyst wall varies from 0.0076 to 0.0102 in thickness (0.0089), consisting of a thin inner and a much thicker outer membrane. Through transparent cyst wall the larva can be readily observed. The larva in the fully developed cyst bends its body in various positions and is actively motile. When the cysts are removed from the crab and put in water or in normal saline, part of larvae may emerge from one end or from the side of cyst wall in 5 to 15 minutes at a room temperature of 28°C. Many larvae die however before leaving cyst wall.

Excysted larva (based on 20 specimens fixed in 2% formalin) is 0.4437 to 0.6090 long by 0.2538 to 0.3066 wide (0.5786 x 0.2813). Oral sucker is 0.1175 long by 0.1146 wide; acetabulum situated in middle third of body, much smaller than oral sucker, 0.0841 long by 0.0754 wide. Entire body is covered with minute spines. Internal structures of excysted larva are similar to those of adult except for the poorly differentiated genital organs. Digestive tract consists of a prepharynx, an elliptical pharynx (0.0498 long by 0.0343 wide), a slender esophagus (0.0803 long) and two short, stumpy intestinal ceca (left, 0.1445 long; right, 0.1518 long). Prepharynx can not be observed readily owing to the presence of large oral sucker. Excretory bladder is elliptical, containing colorless granules. Flame cell pattern is 2(1+2+2+1+1). Most of the genital organs are well developed. Testes are round or ovoid, 0.0908

in transverse diameter by 0.0878 in longitudinal. Cirrus sac an oval ring, 0.1271 in length diameter by 0.1488 in width. A tubular seminal vesicle and prostate glands can be observed in the sac. Ovary 0.0528 long and 0.0462 wide. Oviduct, vitellaria and uterus can not be observed distinctly.



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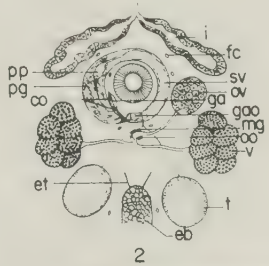

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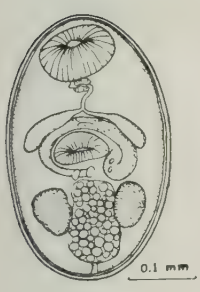

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Plate II

All drawings except Fig. 2 were made with aid of a camera lucida.

Fig. 1. Adult worm of *M. tamsuiensis*, dorsal view.

Fig. 2. Schematic drawing of reproductive organs of *M. tamsuiensis*, ventral aspect.

Fig. 3. Uterine eggs of *M. tamsuiensis*, from microscopic section.

Fig. 4. Excysting larvae of *M. tamsuiensis*.

Fig. 5. Metacercaria of *M. tamsuiensis*.

Abbreviations: co, chitinous opening; eb, excretory bladder; et, excretory tubule; fc, flame cell; ga, genital atrium; gas, genital accessory organ; i, intestine; mg, Mehlis' glands; oo, oötype; ov, ovary; pg, prostate glands; pp, pars prostatica; sv, seminal vesicle; t, testis; v, vitellaria.

FEEDING EXPERIMENTS WITH METACERCARIA

Feeding experiments were carried out in mice, rats, chickens and duckling. Adult worms were recovered from the small intestine of ducklings 4 days after feeding. Although numerous metacercariae were fed to duckling, very few adults were obtained. It seems safe to assume that the duckling is not the optimal final host for this fluke. Eggs of this parasite were not found in feces of the infected animals. Excysted larvae and immature worms were found in the digestive tract in all the experimental animals. A nearly mature but dead worm was recovered from one rat. In the mouse the larvae usually disappeared in 2 days after feeding.

Normal development of the genital organs of this parasite occurred only in the duckling. Within 24 hours after feeding, 7 vitelline follicles on each side of body were clearly visible. Testes and ovary also developed rapidly; however, neither spermatozoa nor eggs were noted at end of 24 hour period. Excretory bladder was more distinct since it was filled with irregular dark granules; flame cells were visible in pattern given above. Within 48 hours after feeding, vitelline ducts were visible, seminal vesicle was filled with flagellated spermatozoa, and a few immature eggs were seen in uterus. No marked changes in the excretory system. In 72 hours after feeding, immature eggs increased in number and were seen in the entire length of tortuous uterus. Four days after feeding, the worm became mature and distal portion of uterus was filled with mature eggs.

Young moscovy ducks (*Cairina moschata* L.), domestic ducks (*Anas platyrhyncha var. domestica* L.) and mule ducks were employed as experimental hosts. The mule duck is the hybrid between male moscovy duck and female domestic duck. Although these ducks belong to the same family, the larva of this fluke developed to adults only in moscovy and mule ducks.

SUMMARY

In October 1959, an undescribed species of metacercaria was recovered from two species of river crabs, *Sesarma bidens* and *Sesarma plicatum*, collected from the Tamsui River in northern Taiwan. The incidence of the metacercaria in the crabs were 74.6% and 80.9%, respectively. After experimental feedings of the metacercariae to mammals and birds, adult worms were obtained from the small intestine of ducklings. A careful study of the flukes showed them to represent a new genus and a new species in the family *Microphallidae*. A new genus (*Macrostomtrema*) and species (*tamsuiensis*) was established, the latter named after the Tamsui River where the crabs were collected.

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Observations on the Life Cycle of *Echinostoma pinnicaudatum*, n. sp., (Echinostomatidae: Trematoda)

P. NASIR*

Five *Lymnaea stagnalis* (L.), supposedly uninfected or in which infection had not reached the stage at which cercariae could be discharged, were left in a glass tank and examined after two weeks in October, 1956. Two of the snails were shedding cercariae of *Cotylurus brevis*, one was shedding *Cercaria ocellata* and two were negative. One of the "negatives" was dissected revealing the presence of tetracotyliform larvae in liver and a certain number of unknown echinostome cysts in renal organ; some of those cysts were recently formed since the contained metacercariae were active. It seemed probable that these cysts were formed by echinostome cercariae which emerged either from the three above cited snails or the fifth one which was yet to be examined. All of the three snails with furcocercous infection proved negative for echinostome cercariae but harbored a variable number of echinostome cysts in their renal organs. Thus the fifth snail, in prevailing circumstances, could have been the only carrier of this particular echinostome infection. On removing a part of its shell, there emerged numerous echinostome cercariae with a fin fold on tail. The rest of the shell was removed very carefully so that the snail was extracted without any apparent injury. It was placed with seven laboratory-bred *L. stagnalis* in a dish to obtain encysted cercariae for experimental feeding. On the second day its digestive gland and hermaphrodite organ were missing, leaving the foot and associated viscera intact. It seemed likely that the organs in question being softer than the foot might have been scavenged by the other snails. The remaining snail-tissues yielded an ample supply of rediae and cercariae, for morphological study, from the pulmonary chamber and adjoining parts; in addition sixty-four cysts were collected from the renal organ and stored in refrigerator.

A week later the seven laboratory-bred snails which had been exposed to echinostome cercariae were dissected and searched for cysts. Although all these snails were infected, they harbored a very small number of cysts; maximum seventeen in one of the snails. Previous experience with feeding experiments with cysts of cercariae of *Echinostoma nudicaudatum* Nasir, 1960, had shown that when the dosage was small, no adult worms were recovered. Under these circumstances, it was considered expedient to supplement the cysts from laboratory-bred snails with sixty-four cysts collected from the renal organ of the snail which, itself, harbored the echinostome cercariae. It was realized that these cysts could possibly have been present in the snail before it was brought into the laboratory but, at the same time, thought to be very unlikely. Furthermore, the cysts themselves could be distinguished by their size from the only other echinostome cysts encountered in the present investigation of larval trematodes from Edgbaston Pool, e.g., cysts of cercariae of *E. nudicaudatum*. The necessity of repeating these experiments has been fully realized.

All the cysts were fed to a laboratory-bred pigeon about three months old, and echinostome eggs appeared in feces on the fourteenth day after the intro-

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duction of cysts. On the fifteenth day, the pigeon was dissected and nine adult echinostomes were recoverd, six from the large intestine and three from the rectum.

CERCARIA of *Echinostoma pinnicaudatum*, n. sp. (Figs. 1-2)

Measurements in mm are based on ten living specimens naturally escaped from their host.

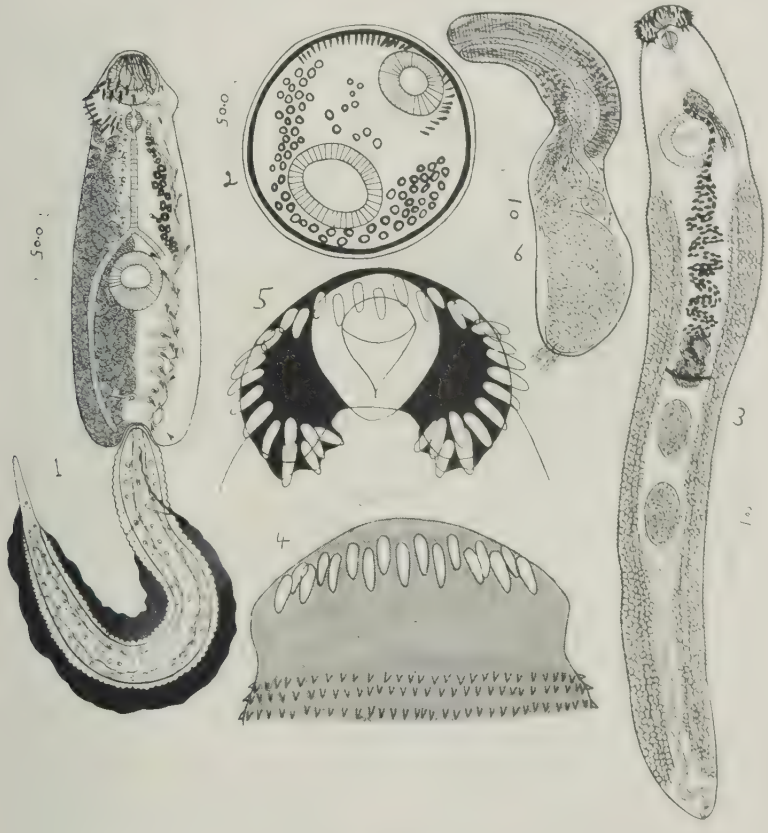
DESCRIPTION: Body, 0.160-0.544 by 0.104-0.184. Tail, 0.160-0.400 by 0.032-0.055, with dorsoventral fin fold arising from proximal end of tail and stopping short of its distal extremity; naked posterior end invaginable and devoid of central caudal strand. Tail and preacetabular region of body beset with transverse rows of spines. Collar spines, 37, arranged in (3 + 2) + 5 + (8 + 9) + 5 + (3 + 2) fashion. Oral sucker, 0.056-0.057 in diameter, ventral sucker 0.056-0.073. Prepharynx, 0.024-0.027 long and pharynx about same in anteroposterior diameter. Esophagus and intestinal ceca filled with a linear series of faintly demarcated masses. Eight terminal ducts at anterior end, probably leading from penetration glands. Cystogenous glands, each with rounded nucleus and granular cyst material. A series of uninucleated bodies, ventral in position, having no connection with terminal ducts, arranged along esophagus and laterally around oral sucker; when treated with neutral red, secretion of these bodies forming an uninterrupted streak. Genital primondia, two masses of cells, one anterior and other posterior to ventral sucker. Excretory system, as shown in diagram; secondary excretory tubes ciliated; flame-cell formula 2 (3 + 3 + 3) + (3 + 3 + 3 + 3 + 3) = 48. Development, in typical echinostome rediae with undivided collar, saccate gut and a pair of lateral appendages. Encystment, in *L. stagnalis;* cysts, 0.147 (0.136-0.182). Host, *L. stagnalis.*

RELATED SPECIES: cercaria of *Echinostoma revolutum* (Froel) Beaver (1937) Johnston and Angel (1941), *Cercaria helvetica XXIV* (Dubois, 1929), "unnamed cercaria" (Johnston and Muirhead, 1949) and *C. cuneata* (Fain, 1953) are the only four echinostome cercariae with a definite number of thirty-seven collar spines and a fin-fold on tail. The cercaria of *E. revolutum* possesses a fin-fold which is limited only to the posterior region of the tail whereas in the cercaria of *E. pinnicaudatum* the dorsoventral fin-fold continues from the base of the tail to almost its posterior extremity. Moreover, in cercaria of *E. revolutum* the fin-fold is confined only to dorsal surface of the tail. *C. helvetica XXIV* has been shown by Beaver to be identical with the cercaria of *E. revolutum.* In the unnamed cercaria of Johnston and Muirhead, the fin-folds at base of the tail are independent of the fin folds at the distal region of tail while in the cercaria of *E. pinnicaudatum* fin folds on dorsal and ventral surfaces of the tail are uninterrupted (excepting a distance short of the extreme posterior extremity). *C. cuneata* has a group of four angle spines while cercaria of *E. pinnicaudatum* has a group of five angle spines.

The cercaria of *Echinostoma lindoensis* (Sandground and Bonne, 1940) is inseparable from the cercaria of *E. pinnicaudatum* on the basis of the nature of fin fold and in point of size. Sandground and Bonne failed to determine the pattern of and exact number of collar spines and they attributed this failure to the smaller size or inconspicuous nature of spines. On the other hand, in cercaria of *E. pinnicaudatum* the spines are neither frail nor so inconspicuous as to obscure their number and arrangement. Except for this difference in the nature of cephalic spination, the two cercariae are indis-

tinguishable. However, as will be shown later, the adult *E. lindoensis* is a distinct species from *E. pinnicaudatum*.

Cercaria limbifera (Seifert) Brown (1931) Rees (1932) and *C. spinifera* (LaVal) Wesenberg-Lund (1934) Ahmed (1959) are the two other echinostome cercariae with a fin fold identical with that of cercaria of *E. pinnicaudatum* but their pattern of cephalic spination is controversial. Seifert (1926) in his fragmentary original account describes thirty-seven collar spines whereas Brown not only in the cercaria but also in excysted immature stage of *C. limbifera* describes thirty-five collar spines. Rees in a detailed study of *C. limbifera* also reports thirty-five collar spines. According to Brown only



Scale in mm.

Fig. 1. General anatomy of cercaria of *Echinostoma pinnicaudatum:* collar spines, flame cell system and cystogenous glands cells shown on one side only.

Fig. 2. Encysted cercaria.

Fig. 3. Adult *Echinostoma pinnicaudatum.*

Fig. 4. Cephalic spination, dorsal view.

Fig. 5. Cephalic spination, ventral view.

Fig. 6. Reconstruction of cirrus sac.

the posterior two-thirds of the tail of *C. limbifera* is marked with a fin fold while in cercaria of *E. pinnicaudatum* dorsoventral fin fold continues to the base of tail. According to Wesenberg-Lund there are forty to forty-five collar spines in *C. spinifera* whereas Ahmed, who bred out the larvae into adult *Echinoparyphium spiniferum* describes thirty-seven collar spines. Even if *C. spinifera* is established as a thirty-seven-spined species it develops into an adult which belongs to a different genus indeed.

ADULT of *Echinostoma pinnicaudatum*, n. sp. (Fig. 3-6)

Based on nine egg-discharging adults fixed in hot Gilson's fluid. Measurements in mm.

DESCRIPTION: Body: 5.528-7.064 by 0.816-1.056; average ratio of breadth to length 1:6.6; cuticle with transverse rows of spines; ventrally, spination extending to about halfway in posttesticular space; dorsally, limited to about halfway in preacetabular region. Head collar, 0.416-0.488 in transverse diameter, with thirty-seven spines continuous dorsally but interrupted ventrally in pharyngeal region and arranged in the (3 + 2) + 5 + (8 + 9) + 5 + (3 + 2) fashion. Of five corner spines innermost oral corner spine 0.055-0.061 long, middle oral corner spine 0.063-0.068 and lateral oral corner spine 0.061-0.068; inner aboral corner spine 0.057-0.061 long and lateral aboral corner spine 0.070-0.079. Five unalternating lateral spines 0.061-0.074 long. Of alternating dorsal series eight orals 0.061-0.072 long, nine aborals 0.066-0.072 and median aboral 0.066-0.077. Oral sucker, 0.20-0.24 in diameter. Ventral sucker, 0.480-0.576 by 0.520-0.584, at distance of 0.608-0.720 from posterior border of oral sucker; cuticular lining of acetabular orifice thrown into indentations. Prepharynx, 0.008-0.035 long. Pharynx, 0.152-0.189 by 0.136-0.162; ratio of pharynx to that of oral and ventral suckers 1:1.3:3.5. Esophagus, 0.232-0.352 long, bifurcating at a distance of 0.120-0.200 from anterior border of ventral sucker; distance between posterior terminations of intestinal ceca and posterior end of body, 0.152-0.296. Testes, tandem, entire, anteroposteriorly elongate, in first third of posterior half of body; anterior testis slightly smaller than posterior testis, 0.400-0.560 by 0.224-0.312, at a distance of 1.528-2.056 from posterior border of ventral sucker; posterior testis, 0.432-0.648 by 0.224-0.280, at a distance of 1.80-2.344 from posterior end of body; intertesticular space, 0.016-0.224. Cirrus sac, muscular, 0.336-0.368 by 0.152-0.248, pyriform, in postbifurcal space, median or to one side of middle line, never extending posterior to equator of ventral sucker; seminal vesicle, with swollen basal part and coiled narrow anterior tubular region; voluminous pars prostatica and profuse prostatic cells present; cirrus, muscular, unspined. Ovary, spheroidal, 0.184-0.256 by 0.232-0.296, median, at distance of 2.424-3.104 from anterior end of body; distance of ovary from anterior testis and posterior border of ventral sucker, 0.176-0.328 and 1.36-1.568 respectively; Mehlis gland, Laurer's canal, receptaculum seminis and receptaculum seminis uterinum present; in most egg-laying adults uterine coils from 9-10; eggs, yellowish, operculate, measurements of extrauterine eggs, 0.097-0.115 by 0.067-0.072. Common genital pore, median or slightly dexteral, ventral to esophageal bifurcation, at a distance of 0.040-0.059 from anterior border of ventral sucker. Vitelline follicles, extending from ventral sucker to almost posterior end of body, never extending to equator of ventral sucker; fields of two sides distinctly apart, non confluent in posttesticular space. Excretory system similar to that of *Echinostoma nudicaudatum* Nasir, 1960.

Discussion

In determining the specific identity of *Echinostoma pinnicaudatum* characters like number of collar spines, relative size of various spines, their arrangement, length of uterus, relative size of suckers and pharynx as outlined by Beaver (1937) in his study on *E. revolutum*, have proven to be of great importance. To these characters may be added the lobed or unlobed condition of testes in adults and characteristics of cercarial stages where life histories are known.

There are ten thirty-seven spined species (see Nasir, 1960) in the genus Echinostoma and *E. pinnicaudatum* resembles only two of these, namely, *E. lindoensis* (Sandground and Bonne) on the basis of cercarial characters and *E. nudicaudatum* (Nasir) on the basis of adult characters.

The cercariae of *E. lindoensis* and *E. pinnicaudatum* are almost indistinguishable whereas the adults are distinct species in the following characters: the largest spine in *E. lindoensis* is 0.095 in contrast with 0.079 in *E. pinnicaudatum;* moreover the largest spine in *E. lindoensis* belongs to unalternating lateral series and in *E. pinnicaudatum* it is the lateral aboral spine of the corner group. As mentioned by Sandground and Bonne "the testis (of *E. lindoensis*) are almost invariably deeply lobed and in gravid specimens the testes are already posette or clover-leaf in shape" while the testes in *E. pinnicaudatum* are very definitely unlobed.

Adult specimens of *Echinostoma pinnicaudatum* resemble very closely those of *E. nudicaudatum* the only distinction being a difference in the arrangement of collar spines. In *E. pinnicaudatum* there are five unalternating lateral spines but in *E. nudicaudatum* there are seven of these. While recognizing this difference, it must be pointed out that as a result of histological processing, the relative disposition of the cephalic spines may become disturbed. Identification is reliable only after treatment which avoids flattening of specimens, e.g. fixation by free immersion in the fixative. Such fixation often results in inconvenient rolling or curling of specimens but these effects may be avoided by employment of hot fixative, e.g. by fixing specimens into Gilson's fluid warmed up to 62° C. Except for the above mentioned difference in the arrangement of collar spines, *E. pinnicaudatum* and *E. nudicaudatum* bred out in pigeons from their larval stages found in *L. stagnalis* in Edgbaston Pool, are very much alike, whereas their cercarial stages are clearly distinct: in the cercaria of *E. pinnicaudatum* there is a well-developed fin fold but in the cercaria of *E. nudicaudatum* the fin fold is absent altogether. Thus a new name *Echinostoma pinnicaudatum* (Latin, *pinna,* fin; *cauda,* tail) has been proposed for this species.

Specific diagnosis

Genus: *Echinostoma* Rudolphi, 1809 *Sensu* Dietz (1909, 1910) emend. Mendheim 1943).

Species: *Echinostoma pinnicaudatum*, n. sp. Head collar with thirty-seven spines and the length of these spines 0.055-0.079, arranged in the (3 + 2) + (5) + (8 + 9) + 5 + (3 + 2) fashion; five corner spines on each side of pharynx, with innermost oral spine the shortest, and the lateral aboral spine of the corner group the largest of the whole series of thirty-seven spines; seventeen unpaired dorsal spines with eight orals only slightly shorter than nine aborals. Testes unlobed. The cercaria with a well-developed dorsoventral fin fold excepting extreme posterior extremity of tail; the number

and arrangement of collar spines identical with that of the adult; cystogenous glands with granular contents.

SUMMARY

Echinostoma pinnicaudatum a thirty-seven spined species was reared in a laboratory-raised pigeon from the cercarial stage found in *Lymnaea stagnalis*. The cercaria is characterized with a dorso-ventral fin fold on its tail except for a short distance posteriorly and granular contents of cystogenous gland cells.

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A new Species of the Genus *Paurodontus* Thorne, 1941, (Nematoda: Neotylenchidae) from India

M. RAFIQ SIDDIQI*

During the author's investigations on the plant-parasitic nematodes of Aligarh (U. P.), India, some nematodes were collected in March, 1957, from soil around cabbage roots. On study these were found to belong to the genus *Paurodontus* Thorne, 1941, and appeared to be very similar to *P. gracilis* Thorne, 1941. Closer examination, however, revealed that they represent a new species for which the name *P. similis,* n. sp., is being proposed. The species is fully described below. The worms were of normal body texture and when freshly removed from soil were very active, showing vigorous movements like the free-living soil nematodes.

The genus *Paurodontus* was established by Thorne in 1941 under a new sub-family, Paurodontinae, which is characterized by the presence of a stem-like extension of the basal oesophageal bulb. Thorne (l. c.) described four new species in this genus from soil around plant roots in North America. Since then, no additional species have been reported. These species show a great diversity in structure which may possibly lead to the splitting of the genus when more species are added.

Paurodontus similis, n. sp. (Fig. 1, A-E)

MEASUREMENTS: 6 females: Length = 0.68-0.75 mm. (0.7 mm.); a = 32-40 (34.6); b = 6.6-7.5 (7.1); c = 9.5-10.5 (10); V = 79-83.5% (80.6%); spear = 9-10 microns.

4 males: Length = 0.52-0.64 mm. (0.57 mm.); a = 34-36 (35); b = 5-6.3 (5.6); c = 8.7-9.6 (9.2); T = 45-50% (48%).

FEMALE (Holotype): Length = 0.68 mm.; a = 40; b = 7.2; c = 10.2; V = $^{41\cdot80}\%^{\cdot3.5}$.

Body elongate, cylindrical, tapering regularly from level of base of oesophagus to lip region and posteriorly behind region of vulva to an elongated, sub-acute tail. Head about three-sevenths as wide as body at base of oesophagus. Body striae distinct, averaging 1.3 microns apart on mid-body, completely interrupted by lateral fields; latter marked by four distinct incisures. Deirids located at level of excretory pore (Fig. 1, B). Phasmids situated slightly over one anal-body-diameter behind level of anus.

Lip region low, flattened anteriorly, marked by three distinct striae, continuous with body contour, 7.5 microns wide at its base and 3 microns high. Labial frame-work moderately sclerotized, hexa-radiate. Spear 9.5 microns long, divisible into two equal parts. Basal knobs of spear rounded, 2.5 microns across. Orifice of dorsal oesophageal gland close to spear base. Corpus a cylindrical tube, with a weakly developed basal swelling. Isthmus long, crossed by nerve ring near its posterior end. Basal oesophageal bulb rather spindle-shaped, with three gland nuclei and a short stem-like extension that projects into the lumen of the anterior end of the intestine. Excretory duct distinct, opening at level of oesophageal base through a cuticularized pore. Hemizonid three body annules long, situated just anterior to excretory pore.

Ovary single, outstretched, with oöcytes in a single file. Uterus long, highly extensible. Posterior uterine branch sac-like, about one-and-a-half times vulvar-body-width long. Vagina at right angles to body axis, extending

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about one-third into the body. Vulva a depressed transverse slit. Vulva-anus distance greater than tail length. Tail regularly tapering to a sub-acute, rounded terminus, slightly less than seven anal-body-diameters in length.

MALE: (Allotype): Length = 0.64 mm.; a = 35.7; b = 5.8; c = 9.6; T = 50%.

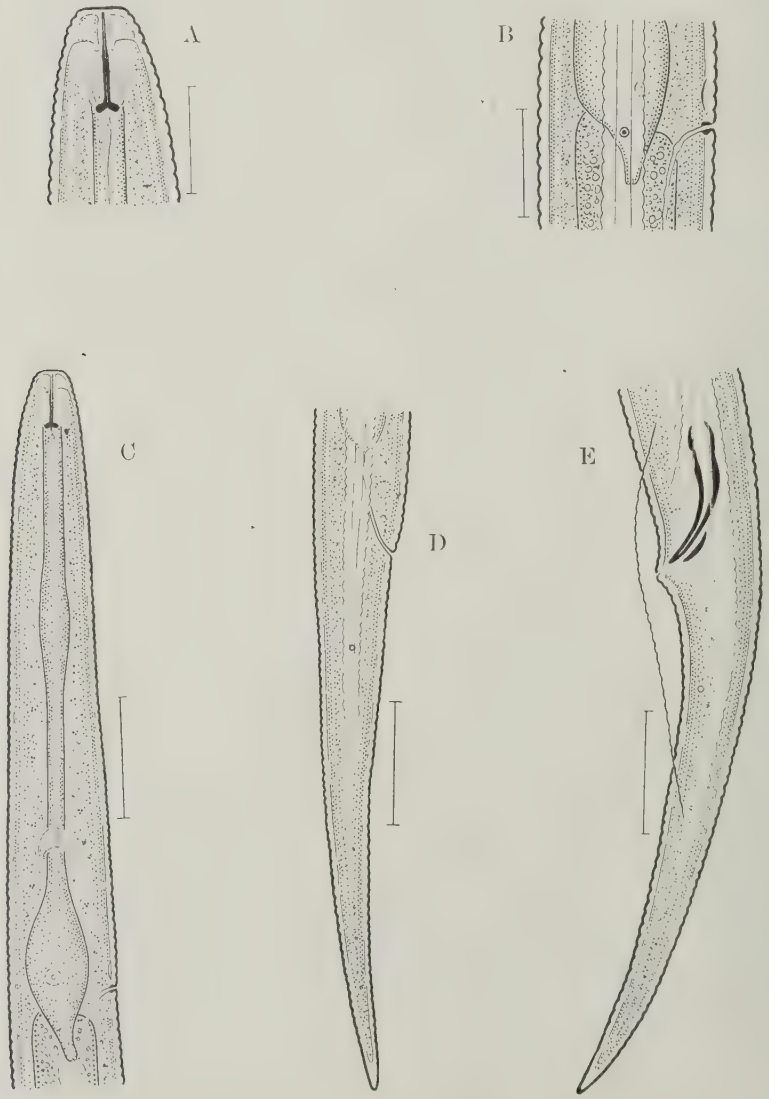

Figure 1. *Paurodontus similis*. A. Head of female. B. Oesophageal base of female. C. Oesophageal region of male. D. Female tail. E. Male tail. Scales: A and B = 10 microns; C = 20 microns; D and E = 15 microns.

Body essentially similar to that of female. Transverse striae 1.2 microns apart on mid-body. Lip region with three transverse striae, supported by moderately sclerotized frame-work. Basal oesophageal bulb with a stem-like basal extension. Intestinal cells with rounded, refractive granules.

Testis single, outstretched; spermatocytes serially arranged. Spicules of medium strength, ventrally arcuate, cephalated, 19 microns long. Gubernaculum simple, trough-shaped, 6 microns in length. Bursa distinctly crenate, about three-and-a-half times anal-body-width long, enveloping about one-third of tail. Tail elongate, regularly tapering, slightly ventrally arcuate, ending in a sub-acute rounded terminus.

HOLOTYPE: Female collected on March 3, 1957; tube no. PN/N/2-001; deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U.P.), India.

ALLOTYPE: Male; tube no. PN/N/2-002; other data same as for holotype.

TYPE HOST: Collected from soil around roots of cabbage, *Brassica oleracea* L.

TYPE LOCALITY: Aligarh (U. P.), India.

DIAGNOSIS AND RELATIONSHIP: *Paurodontus* with the above measurements and general description. It is distinguished by the following characters: Elongate, slender body; presence of three striae on the head and distinct deirids at the level of the excretory pore; position of the vulva at 79-83.5 per cent of the body length from the anterior end; presence of a post-uterine sac which is one-and-a-half times vulvar-body-width long; bursa enveloping one-third of the male tail; tails of both sexes elongate, slightly less than seven anal-body-diameters long, ending in a sub-acute terminus.

P. similis, n. sp., is closest to *P. gracilis* Thorne, 1941, from which it is easily distinguished by the absence of the peculiar chamber surrounding the basal oesophageal bulb, a more posteriorly located vulva (Vulva at 76 per cent of body length in *P. gracilis*), vulva-anus distance being greater than tail length, a shorter tail with a sub-acute rounded terminus (c = 9.5-10.5: 7.2) and the bursa enveloping one-third of the male tail.

A KEY TO THE SPECIES OF PAURODONTUS (BASED ON FEMALES)

1. Body-length about 0.7 mm.; stem-like basal extension of oesophagus short; tail elongate, about 7 × anal-body-width long 2
   Body-length about 0.4 mm.; stem-like basal extension of oesophagus long; tail conoid, about 3-4 × anal-body-width long 3
2. Oesophageal bulb enclosed in a chamber; c = 7.2, tail terminus acutely pointed ..... *P. gracilis* Thorne, 1941
   Oseophageal bulb not enclosed in a chamber, c = 9.5-10.5, tail terminus sub-acutely rounded ..... *P. similis*, n. sp.
3. Tail ventrally arcuate, sub-acute; vulva-anus distance greater than tail length ..... *P. densus* Thorne, 1941
   Tail straight, sharply pointed; vulva-anus distance less than tail length 4
4. Head two-fifths as wide as body at base of oesophagus, a = 16-22, post-uterine branch absent ..... *P. apiticus* Thorne, 1941
   Head three-fifths as wide as body at base of oesophagus, a = 24, post-uterine branch present ..... *P. niger* Thorne, 1941

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Longidorus menthasolanus, a New Plant Parasite from Oregon (Nemata: Dorylaimoidea)*

DONALD E. KONICEK and HAROLD J. JENSEN**

Longidorus menthasolanus, n. sp., is a serious pest of peppermint in certain areas of Oregon (Horner and Jensen, 1954, and Jensen and Horner, 1956). Approximately 1,000 of the 14,000 acres of peppermint in the state were estimated to be infected in 1958 (Jensen, 1958). Damage in individual fields ranges from a trace to the development of large barren areas which may on occasions envelop an entire field. Preliminary host range trials indicate this species is also a potential pest of many solanaceous plants. The nematode was first reported to be *Longidorus sylphus* Thorne, 1939 (Jensen and Horner, 1956) but recent studies indicate several morphological differences exist.

Longidorus menthasolanus, new species

MEASUREMENT: 30 females: Length = 5.1 mm. (3.9-6.1); a = 78 (61-101); b = 13 (10-16); c = 105 (77-134); V = $^{8}48\%^{8}$ ($^{3\text{-}13}44\text{-}50\%^{4\text{-}14}$); spear = 89 microns (70-110); extensions = 60 microns (30-68).

MALE: 30 males: Length = 4.6 mm. (3.6-5.1); a = 97 (66-121); b = 21 (12-33); c = 98 (72-136); (10 males T = 48% (33-70); spear = 89 microns (65-98); extension = 60 microns (41-62).

FEMALE (Holotype): Length = 4.2 mm.; a = 81.5; b = 10.4; c = 113.; V = $^{5.4}147.9\%^{7.2}$: spear = 70.5 microns; extensions = 45 microns.

MALE (Allotype): Length = 4.5 mm.; a = 106.7; b = 17.2; c = 100.8; t = 52.9%; spear =72 microns; extensions = 46 microns.

DESCRIPTION: Body long and slender, tapering towards anterior extremity, curving into gentle arch when relaxed with heat. Lip region expanded slightly, forming a semi-knob-like head. Six lips having two circlets of six and ten papillae. Amphids abnormally large, almost encircling head. Amphid apertures slit-like, usually obscure. Guiding ring near apex of spear. Spear length variable (65-110 microns) with slight bow in posterior one-third. Spear extensions (30-68 microns) ranging from one-half to two-thirds as long as spear. Esophagus beginning as a slender tube which expands rather abruptly into an elongated basal bulb. Bulb length equal to or slightly greater than that of spear. Cardia bluntly conoid. Intestine six cells in circumference.

FEMALE: Vulva a transverse slit located near middle of body. Ovaries short and reflexed one-half or more their length. Eggs when present approximately two and one-half times body width and three times as long as wide. Prerectum from ten to eleven times anal body width. Rectum length about equal to anal body diameter. Two pairs of caudal papillae present, and one preanal pair directly opposite rectum in median location. Tail with conspicuous radial striae, bluntly convex-conoid, slightly longer than anal body diameter.

MALE: Testes often lacking or only partially developed with granular bodies resembling sperm present. Supplements consisting of an adanal pair and six to ten in a ventro-median series, beginning within range of the

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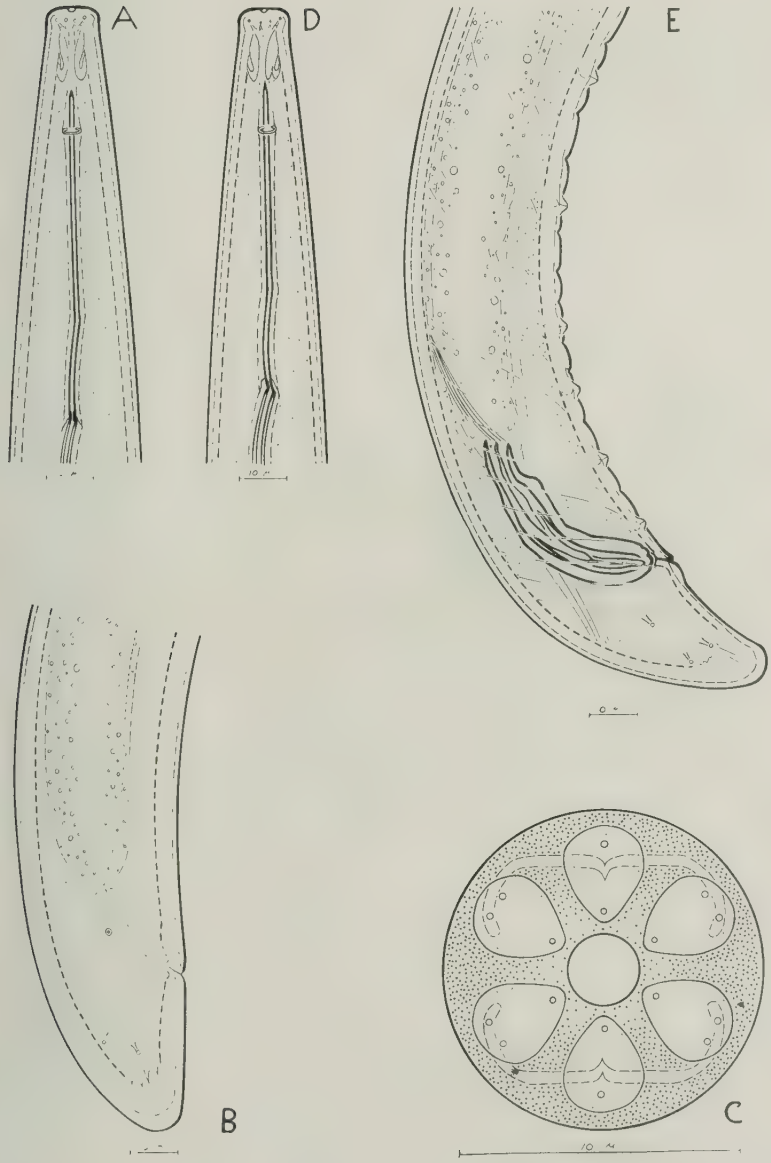

Figure 1. *Longidorus menthasolanus*, n. sp. A. Female anterior portion. B. Female posterior portion. C. Female face view. D. Male anterior portion. E. Male posterior portion.

spicula and spaced uniformly. Prerectum seven to eight times anal body width. Spicula blunt, arcuate, and with small prong branching from each side of proximal extremity to three-fourths of its length. Small lateral guiding pieces present. Oblique copulatory muscles prominent. Six caudal papillae present, two sub-ventral, two subdorsal, and two located in a median or lateral position, tail with conspicuous radial striae, bluntly convex-conoid, length slightly longer than anal body diameter.

Diagnosis: *Longidorus menthasolanus* can be separated from two other closely related species, *L. sylphus* Thorne, 1939 and *L. elongatus* Thorne and Swanger, 1936, by the morphological features given below:

In *L. menthasolanus* the lip region is set off slightly, the tail is bluntly convex-conoid (as compared to dorsally convex-conoid to blunt terminus) and males are present. It may be separated from *L. sylphus* on these bases.

Longidorus menthasolanus differs from *L. elongatus* as follows: The lip region or head is not set off as much; body width is greater in proportion to body length; female tail is generally shorter; male tail is bluntly convex-conoid and is not tapered as much.

Holotype: Female collected on September 3, 1958; slide deposited with Department of Botany, Oregon State College, Corvallis, Oregon.

Allotype: Male collected on September 3, 1958; slide deposited with Department of Botany, Oregon State College, Corvallis, Oregon.

Paratypes: Twenty-nine females and twenty-nine males collected from soil about roots of mint; other data same as for holotype and allotype.

Type Host: Collected from soil around roots of peppermint, *Mentha piperita* L.

Type Locality: Talbot, Oregon, United States of America.

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Report of the Brayton H. Ransom Memorial Trust Fund

Funds On Hand, Jan. 1, 1960		$1933.30
Receipts: Interest rec'd in 1960		67.92
Contribution to principal		5.00
Disbursements: Grant to Helminthological Society of Washington	$10.00	
Expenses, Brayton H. Ransom Award	37.30	
		47.30
Balance On Hand, Dec. 31, 1960		$1958.92

A. O. Foster
Secretary-Treasurer

Skin penetration by infective larvae of *Placoconus lotoris*

S. P. GUPTA[1]

Skin penetration of infective larvae of most hookworms has been known for a long time. However no work has been done so far to determine whether or not the ensheathed larvae of *Placoconus lotoris* (Schwartz, 1925) Webster, 1956 from a raccoon (*Procyon lotor*) and the skunk (*Mephitis mephitis*) are skin penetrators. In view of its importance as a parasite of fur bearing animals, a series of experiments were undertaken to determine whether or not the larvae were able to infect the host through the skin.

MATERIALS AND METHODS

The material used in the present studies was originally obtained from a raccoon, received from U. S. Department of the Interior Fish and Wildlife Service.

The faeces of a raccoon with equal quantities of helminthologically sterile sand and charcoal were made into a moist culture with tap water. Eggs of *P. lotoris* from the raccoon were teased from the uterus or released by grinding the female in sand and similarly cultured in rabbit faeces and charcoal at room temperature (22° to 24°C.). The cultures were maintained in petri dishes, the lids of which were lined with filter paper. After 5 days, infective larvae were recovered from the cultures by Baermann technique. This method provided numerous vigorous larvae.

The actual penetration was studied in the manner described by Goodey. A young mouse (body about 1 inch long) was chloroformed and the skin from the abdomen and flanks removed. This skin, hair upwards, was stretched and pinned over a hole about ¾ inch in diameter in the centre of a piece of a cork sheet about 1/6″ in thickness. The cork was then floated on the surface of physiological saline at 37°C in a small beaker, about 2 inch in length and 1½″ in diameter. To be certain that the warm saline came into contact with the underside of the skin precautions were taken to allow any bubbles of air to escape from between the skin and the cork.

The entire preparation was then placed in the incubator room at 37°C on the stage of binocular dissecting microscope. A drop of water containing a large number of active infective larvae were placed on the skin. Immediate examination showed that the larvae were active and were wriggling downwards with their anterior ends pressing against the skin, as if trying to penetrate through it.

After a couple of hours, it was observed that the drop of water had evaporated and the majority of larvae were found moving in the saline beneath the skin. In order to determine if there were any sheaths left on the skin, a drop of water was placed over the dried skin. A large number of empty sheaths and a few actively moving larvae were found. It seems evident that the larvae had penetrated the skin, although actual penetration was not observed.

The skin was then fixed in 70% alcohol and the next day, the epidermis was separated from the deeper layers, cleared in lactophenol, and mounted

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The author is grateful to Dr. Calton M. Hermann, of U. S. Department of the Interior Fish and Wildlife Service for providing a racoon required in the present studies

whole. In this way empty sheaths were observed on the surface of the skin and a large number of larvae were found embedded within the epidermis.

From this it is concluded that larvae penetrate skin leaving the sheaths outside.

SUMMARY

Infective larvae of *Placoconus lotoris* are skin penetrators.

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MINUTES

Three Hundred Seventy-Third Through the Three Hundred Eightieth Meetings

373rd meeting: Student Union, University of Maryland, College Park, Maryland, 8 October, 1960. 50th Anniversary Meeting with over 200 members and guests in attendance. Morning program consisted of formal papers as follows: Some dietary factors that affect ovarial transmission of symbiotes by M. Brooks (discussion by T. von Brand); Physiology of intracellular parasites by W. Trager (discussion by J. Andrews). Afternoon program consisted of several concurrent informal discussions as follows: Immunity to parasites—moderated by L. Jackowski, J. Oliver Gonzalez and R. Anderson; Nematodes of plants—moderated by V. Dropkin and M. Allen; Physiology of parasites—moderated by T. von Brand and E. Bueding; Culture of parasites—moderated by P. Weinstein and G. Ball; Systematics and nomenclature—moderated by A. McIntosh and G. LaRue; Chemotherapy—moderated by P. Harwood and G. Otto. Evening program consisted of a banquet, presentation of special Certificate of Recognition to Edna M. Buhrer, presentation of Brayton H. Ransom Memorial Award to James H. Turner and an address by Chauncey D. Leake entitled "Paralogue and Parasite." This meeting was reported in detail in a special Fiftieth Anniversary Issue (Volume 27, Number 3) of the Proceedings.

374th meeting: Log Lodge, Agricultural Research Center, Beltsville, Maryland, 18 November 1960. D. A. Shorb appointed as Representative to Washington Academy of Science. Papers presented; Some observations on the invasion of the liver by infective larvae of the large intestinal roundworm of pigeons, *Ascaridia columbae* by Wehr; Sex difference in susceptibility of hamsters to *Nippostrongylus brasiliensis* by Haley; Excystation of coccidia by Lotze and Leek; Technique for recovering root knot nematodes from plant

tissues by by Dropkin; Recovery of microfilariae and adult filarid parasites from the Colombian marmoset by Sawyer; and *Culicoides furens,* possible vector of the filarid of the Colombian marmoset by Rozeboom.

375th meeting: Sternberg Auditorium, Walter Reed Army Institute of Research, Washington, D. C., 19 December 1960. The following slate of officers was elected: L. E. Rozeboom, President; C. M. Herman, Vice President; A. J. Haley, Recording Secretary; E. M. Buhrer, Corresponding Secretary-Treasurer. Papers presented: Serology of schistosomiasis using minute amounts of dried blood. I. Development of a technique by Anderson, Sadun and Williams; II. Application of the fluorescent antibody test to epidemiologic investigations by Sadun, Anderson and Williams; *In vitro* maintenance of the adult heartworm, *Dirofilaria immitis* by Rothstein; Genetic aspects of the susceptibility of mosquitoes to malarial infection by Ward; and Epizoology of *Trypanosoma cruzi* in Maryland by Herman, Bruce and McMullen.

376th meeting: Auditorium, Naval Medical Research Institute, Bethesda, Maryland, 20 January, 1961. L. J. Oliver was elected Member at Large. Invitation accepted from American Society of Parasitologists to hold joint meeting in Washington, D. C. in June, 1962. Papers presented: Observations on *in vitro* and *in vivo* envelope formation by *Schistosoma mansoni* cercariae by Stirewalt; *Lernaea cyprinacea,* a parasitic copepod of freshwater fishes by Haley; Antigen-antibody relationships in *Dirofilaria immitis* infections of dogs by Jackowski.

377th meeting: Biology Building, Howard University, Washington, D. C., 17 February, 1961. Joint meeting with the Howard University Chapter of the Society of the Sigma Xi. Annual report of Treasurer approved. Committee on local arrangements for June, 1962 joint meeting with American Society of Parasitologists appointed as follows: Anderson, Diamond, Haley, Tromba, Weathersby and Dropkin as Chairman. Papers presented: Reproductive capacity of *Argas persicus* after long periods of starvation by Rajapaksa; Rate of body growth of rats infected with *Trypanosoma lewisi* by Lincicome; Rate of body growth of mice infected with *Trypanosoma lewisi* by Lincicome; Rate of body growth of mice infected with *Trypanosoma duttoni* by Shepperson; Quantitative studies on heterologous sera inducing development of *Trypanosoma lewisi* in mice by Lincicome and Francis; Mitotic rates of rat liver cells under influence of infection with *Trypanosoma lewisi* by Cherrie.

378th meeting: Wilson Hall, National Institutes of Health, Bethesda, Maryland, 15 March, 1961. Expenditure of $25.00 for support of annual Science Fair Program approved. Resolution toHelminthological Abstracts to continue present coverage approved. Papers presented: Survival and development *in vitro* of fourth stage larvae of the nematode, *Nippostrongylus muris* by Sommerville; Effects of *Trichinella spiralis* infections on EMC virus infections in mice by Oliver; Transformation of antigenic type in *Trypanosoma equiperdum* by Cantrell; Diphasic medium for the cultivation of *Entamoeba histolytica* by Diamond; Appraisal of the present status of schistosomiasis in India by Oliver; Comments on helminthology in Australia by Sommerville.

379th meeting: School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland, 28 April, 1961. Expenditure of $50.00 approved for support of annual picnic meeting. L. A. Jackowski appointed Chairman of Anniversary Meeting Committee. R. S. Haberman awarded

IN MEMORIAM

Ellis Edwin McCoy

November 15,1909 — November 11, 1960

Chief of Laboratory Services, New Jersey Plant Industry Laboratory
Member Helminthological Society of Washington since April 25, 1958

Howard Arthur Winter

May 21, 1912—February 21, 1961

Assistant Professor of Biological Sciences, Mexico City College, and Visiting Investigator (Helminthology) Institute of Biology, National University of Mexico
Member Helminthological Society of Washington since February 26, 1958

commendation of the Society for science fair project entitled, "Treatment of Pinworm in Mice." Papers presented: Rous sarcoma in the embryonated egg with special reference to endothelium by Coates; Studies on pathogenicity in *Eimeria acervulina* in chickens by Krassner; Population density influence on changes in the chemical composition of *Hymenolepis diminuta* during its growth in the definitive host by Roberts; Characterization of a protease from *Schistosoma mansoni* by Timms; Sedimentation characteristics of helminth glycogens by Orrell; Carbohydrate dissimilation by *Ascaris* muscle by Saz; Electron transport systems of *Ascaris* muscle by Bueding.

380th meeting: Log Lodge, Agricultural Research Center, Beltsville, Maryland, 20 May, 1961. Annual picnic meeting. Gerald Thorne elected to life membership for long and conspicuous service to the society.

The following were elected to membership at the meetings indicated: 373rd —T. R. Adkins, R. L. Brown, W. L. Bullock, R. M. Cable, W. F. Cantrell, A. L. Cherrie, J. H. Cross, C. J. Ellis, J. H. Greve, J. E. Guthrie, W. S. Hunter, N. Kingston, L. R. Krusberg, D. K. Lawless, T. G. Meade, B. J. Meyers, R. E. Ogren, N. Rothstein, J. T. Self, J. R. Shepperson, D. R. Viglierchio; 374th—V. R. Ferris, S. Kantor, E. L. Nigh, D. H. Reese, W. Dorsman, I. Wood; 375th—H. Berger, M. E. Doscher, D. E. Konicek, J. S. Pankavich; 376th—D. F. Green, F. J. Grundbacher, H. G. Sen, G. B. Solomon; 377th—J. K. Chiu, A. A. de la Cruz, A. C. Cuckler, A. H. Epstein, R. E. Green, R. Harkema, E. C. Jorgenson, A. C. Kane, G. C. Miller, J. P. Porter, N. K. Rajapaksa, B. E. Tilton; 378th—F. A. Adams, T. R. Bello, M. B. Lima, N. A. Minton, J. L. Saunders; 379th—H C. Hechler, R. C. McCrae, G. A. Paxman, J. O. Simaren, E. D. Wagner, A. F. West, S. Wright; 380th—O. J. Dickerson, M. E. Schellberg, Gerald Thorne (life member).

A. James Haley
Recording Secretary

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